THE EFFECT OF OCEAN WARMING ON THE CORAL COMMUNITIES OF HAWAI'I

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This work is dedicated to my parents, Patrick and Margie Massey,

For their continual love and support,

And to the memory of my cousin, Kailey Blake Massey,

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ABSTRACT

The Main Hawaiian Islands experienced unprecedented back-to-back coral bleaching events in 2014 and 2015. This event occurred in the context of the third and largest global bleaching event to date, which occurred from 2014-2017 and affected reefs in every tropical coral reef habitat around the globe. Bleaching disrupts the symbiosis between coral animals and their algal symbionts, and the physiological damage accrued during times of bleaching causes partial or full mortality and widespread events can have lastly impacts on the functional complexity and diversity of reef systems. In an attempt to better understand bleaching and its effects on reefs, my dissertation addressed the following questions: 1) What were the bleaching patterns at Lanikai in 2014 and 2015, and was there any evidence of acclimatization between years?; 2) Were there genomic differences between differentially bleached *Montipora capitata* next to each other on the reef?; 3) Are growth anomalies (GAs) of *Porites evermanni* morphologically and physiologically different?; and 4) How well did a citizen science reef monitoring project describe reef health and bleaching?

Results revealed characteristic differences in species susceptibility and recovery of bleaching patterns at Lanikai and *in situ* degree heating weeks revealed *Porites* and *Pocillopora* colonies acclimatized to thermal stress from 2014 to 2015, bleaching less per unit area during significantly higher thermal stress. Analysis from genome scans of *M. capitata* found no strong underlying signals of selection to explain differential bleaching responses, despite all harboring clade C *Symbiodinium*. *P. evermanni* GAs showed characteristic morphological and physiological differences with larger corallites and less lipid energy reserves than normal tissue. While normally not reproductive, the GAs of *P. evermanni* were found to be extremely reproductive, illustrating there is still more to learn about these anomalous coral growths. And

finally, the citizen science monitoring project provided an opportunity for the community to take part in understanding how climate change is affecting their reef. With the intensity and frequency of coral bleaching events predicted to increase, it is important now more than ever to understand the ecological, physiological, and molecular aspects of bleaching and the implications for the future of coral reefs.

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LIST OF ABBREVIATIONS AND SYMBOLS

MHI = Main Hawaiian Islands

NWHI = Northwestern Hawaiian Islands

NOAA CRW = National Oceanic and Atmospheric Administration Coral Reef Watch

DHW = degree heating weeks

PAR = photosynthetically active radiation

GA = growth anomaly

WE = wax ester

FAME = fatty acid methyl ester

TG = triacylglycerol

FFA = free fatty acid

ST = sterol

PL = phospholipid

CHC method = Coral Health Chart method

PA method = Professional Assessment method

CHAPTER ONE

INTRODUCTION

Coral Reefs and Climate Change

Coral reefs are dynamic ecosystems host to a wide array of biological diversity (Odum and Odum 1955; Connell 1978; Roberts et al. 2002) which provide a multitude of ecological, social, and economic benefits to people around the world (Moberg and Folke 1999). Coral reefs are worth billions of dollars to the global economy as they provide benefits to humans through shoreline protection, fisheries provide a livelihood for many and an important source of food for others, tourism provides local revenue and jobs, and reef organisms contain substances used in pharmaceutical developments. However, the coral reefs of today are facing some of the greatest threats in their history due local stressors and the increasing degree of anthropogenic disturbance, namely the effects of global climate change (Roberts et al. 2002; Hughes et al. 2003, 2018a, 2018b; Pandolfi et al. 2011).

Disturbances, such as storms (Done 1992; Hughes and Connell 1999), disease outbreaks (Santavy and Peters 1997), overfishing (Jackson et al. 2001; Hughes 1994), and increases in nutrients and pollutants (Lapointe and Clark 1993; Littler and Littler 2006) are local stressors threatening the health and longevity of coral reefs. However, the two largest threats to coral reefs globally are increased sea water temperatures and increased ocean acidification (Hughes et al. 2003; Hoegh-Guldberg et al. 2007; Pandolfi et al. 2011). The driving force behind both of these stressors is the increased concentration of carbon dioxide (CO_2) in the atmosphere as a result of anthropogenic burning of fossil fuels. Carbon dioxide traps heat in the atmosphere due to the greenhouse effect and the oceans act as a sink for this source of excess carbon dioxide. Excess carbon dioxide in ocean waters causes the carbonate chemistry of the water to change and alters the pH towards a more acidic state (Orr et al. 2005; Donney et al. 2009), which in turn affects organisms that secrete hard skeletons, such as the calcium carbonate skeletons of corals. While

ocean acidification is a pressing issue for today's oceans, this dissertation focuses on the effects of increased sea water temperatures, and more specifically coral bleaching.

Coral Bleaching - Causes and Consequences

Coral bleaching occurs as a result of thermal stress induced by elevated sea surface temperatures (Coles et al. 1976; Hudson 1981; Hoegh-Guldberg and Smith 1989; Jokiel and Coles 1990; Jokiel 2004) and increases in levels of irradiance or photosynthetic active radiation (PAR) (Fisk and Done 1985; Lesser et al. 1990; Gleason and Wellington 1993; Salih et al. 1998; Marshall and Baird 2000). Additionally, other environmental factors such as water motion (Goenaga and Canals 1990; Gleason and Wellington 1993; Jokiel and Brown 2004), salinity (Coles and Jokiel 1978; Glynn 1991), sedimentation (Glynn 1991; Meehan and Ostrander 1997; Phillip and Fabricius 1993), low temperature (Gates et al. 1992), and increased ultraviolet radiation (UVR) (Gleason and Wellington 1993; Lesser 1997) can lead to and exacerbate bleaching. When bleaching occurs, it disrupts the symbiosis between coral host and dinoflagellate zooxanthellae which dwell within the gastrodermis of coral polyps (Jokiel 2004). The symbiosis is vulnerable when the coral holobiont is exposed to temperatures as little as 1-2 ^{o}C above their summer maximum temperature (Coles et al. 1976; Coles and Jokiel 1977; Glynn and D'Croz 1990; Buddemeier and Fautin 1993; Jokiel and Brown 2004; Donner et al. 2005).

There are a number of theories regarding what cellular process begins the bleaching response, including damage to the D1 protein needed for photosystem II (PSII) repair (Warner et al. 1996; Takahashi et al. 2004, 2009; Hill et al. 2011), damage to the enzyme Rubisco necessary for the dark reactions (Lesser 1997; Jones et al. 1998), and host carbon concentrating mechanisms (CCMs) for retrieval of inorganic carbon from seawater creating a carbon sink for dark reaction inputs (Wooldridge et al. 2014a). All of these sources of error ultimately lead to

photoinhibition of electron transport in the light reactions. Additionally, there is the bacterial bleaching hypothesis, which postulates high temperatures activate virulent genes and allow for easier infection by causative agents (Rosenberg 2004). Regardless of the initial mechanism, there is consensus in the literature that reactive oxygen species (oxygen radicals), which damage coral and algal membranes, proteins, lipids, and DNA are a major contributor to the bleaching process (Lesser et al. 1990; Lesser 1997; Jones et al. 1998; Tchnerov et al. 2004; Weis 2008; Hill and Takahashi 2014; Krueger et al. 2015).

When symbiosis is disrupted, bleaching occurs via loss of functional zooxanthellae in one of two ways. One, corals may expel their zooxanthellae partners leaving the tissue barren of these dinoflagellate cells, or two, the zooxanthellae may lose the photosynthetic pigments in their individual cells (Glynn et al. 1985; Lesser et al. 1990; Lesser 1997; Jones et al. 1998; Hill and Takahashi 2014). Under either circumstance the loss of zooxanthellae or their pigments results in white or lighter tissue, producing the appearance that the coral has "bleached" (Jokiel 2004). The physiological damage accrued during times of bleaching causes a coral to lose its photoautotrophic source of energy, forcing the coral to utilize excess energy to acquire food heterotrophically (Grottoli et al. 2006, 2014; Wooldridge et al. 2014b). Corals can only survive for a short period of time via heterotrophic energy intake. If a coral can't re-acquire zooxanthellae to resume photoautotrophic feeding, then partial or full colony mortality occurs and bare skeleton remains (Baker 2001; Jokiel 2004).

Decreased gonad development and reproductive output have been observed following the stress of bleaching. When corals lose their autotrophic source of food they must utilize energy stores (lipids, proteins, carbohydrates) and/or supplement their nutrients heterotrophically to survive (Grottoli et al. 2006, 2014). Different species vary in their reliance on heterotrophy for

carbon acquisition, and bleaching stress has shown to decrease energy reserves in corals, particularly lipids (Grottoli et al. 2004; Rodrigues et al. 2008). With decreased immunity and defenses from undergoing thermal stress, corals are often more susceptible to diseases following bleaching events (Bruno et al. 2007; Muller et al. 2008; Miller et al. 2009) as virulence of pathogens increases with temperature (Harvell et al. 2002). While many of the common coral diseases are malignant in nature, thermal stress and subsequent bleaching has also been linked to less malignant diseases such as growth anomalies (McClanahan et al. 2009; Cantin and Lough 2014; Mallela et al. 2015).

Coral Defense Mechanisms

Coral hosts and their symbionts have a number of defense mechanisms to combat the effects of bleaching stress. Coral hosts possess fluorescent pigments and mycosporine amino acids to shield their cells from light stress (Dunlap and Shick 1998; Banaszak et al. 2006; Salih et al. 2006), produce stress proteins and antioxidants (Brown et al. 2002a; Barshis et al. 2010; Hawkins et al. 2015), and regulate gene expression of stress response genes (Ainsworth et al. 2008; Barshis et al. 2010; Bellantuono et al. 2011). Symbionts of different clades impart differential levels of thermal tolerance for the coral holobiont (La Jeunesse et al. 2004; Rowan 2004; Berkelmans and van Oppen 2006; Jones et al. 2008; Jones and Berkelmans 2010; Stat and Gates 2011; Hume et al. 2015), although this frequently includes physiological tradeoffs in non-stressful conditions, such as decreased growth and photochemical efficiency (Little et al. 2004; Cantin et al. 2009; Jones and Berkelmans 2011; Cunning et al. 2015a, 2015b).

Thermal tolerance is an essential component of how corals respond and survive during rapid environmental change (Palumbi et al. 2014; Dixon et al. 2015; Kleypas et al. 2016). Corals acquire thermal tolerance via two mechanisms, acclimatization and adaptation. Acclimatization

occurs within a single generation when an individual's physiological response to stress results in a change in phenotype that boosts that individual's tolerance. Adaptation occurs over successive generations within a population and occurs when individuals with stress-tolerant genotypes display relatively greater fitness despite environmental change (Barrett and Schluter 2008; Savolainen et al. 2013).

Corals can acclimatize to thermal stress through changes to coral host physiology and/or algal symbiont type. The literature shows that shifts to a more thermally-tolerant symbiont type via symbiont "shuffling" results in localized acclimatization to thermal stress (Baker et al. 2004; Rowan 2004; Berkelmans and van Oppen, 2006; Jones et al. 2008; Baker et al. 2013; Grottoli et al. 2014; Cunning et al. 2015b; Silverstein et al. 2015). The rise of the use of '-omic' methods has increased understanding of the molecular component of the coral host thermal stress response (Sweet and Brown 2016; Louis et al. 2017). In particular, '-omics' analyses have extensively investigated the acclimatization potential for corals in the future through utilizing existing environmental gradients to investigate the relationship between thermal history and gene expression during thermal stress (Bellantuono et al. 2012; Barshis et al. 2013; Bay et al. 2013; Kenkel et al. 2013a; Palumbi et al. 2014; Bay and Palumbi 2015; Kenkel and Matz 2016; Lee et al. 2018; Thomas et al. 2018). Other studies have identified evidence of adaptation within the coral host genome (Smith-Keune and van Oppen 2006; Bay and Palumbi 2014; Dixon et al. 2015; Kenkel et al. 2015a). Collectively, these studies show that a coral's environment and thermal history play pivotal roles in its' thermal tolerance capacity and future adaptive potential.

Bleaching History

The conditions that elicit thermal stress and ultimately coral bleaching are becoming more prevalent due to the increasing severity of global climate change (Hoegh-Guldberg 1999;

Hughes et al. 2003, 2017, 2018a), and widespread bleaching events are increasing in frequency and severity (Hoegh-Guldberg 1999; Jokiel and Brown 2004; Eakin et al. 2016; Hughes et al. 2017, 2018a). Bleaching was first reported on the Great Barrier Reef in 1931 (Yonge and Nichols 1931). However, bleaching did not become an issue of wide concern until the first major bleaching event in 1983-84, which affected multiple locations across the eastern Pacific (Glynn 1983, 1991; Glynn and D'Croz 1990; Hoegh-Guldberg et al. 1999). There have been many isolated bleaching events since 1983; however, the most expansive bleaching events to date, termed "global bleaching events", took place in 1998, 2010, and 2014-17 (Goreau et al. 2000; Eakin et al. 2016; Hughes et al. 2018a). These global events were related to the influence of global scale oceanographic conditions like the El Nino Southern Oscillation (Philander 1983) and the Pacific Decadal Oscillation (Rodgers et al. 2015). These global bleaching events affected corals on almost every reef around the globe and resulted in significant mortality to corals worldwide. In Hawai‘i specifically, major bleaching events occurred in the main Hawaiian Islands (MHI) in 1996, 2014, and 2015 (Bahr et al. 2015; Eakin et al. 2016); and in the Northwestern Hawaiian Islands (NWHI) in 2002, 2004, and 2014 (Aeby et al. 2003; Jokiel 2004; Couch et al. 2017). The bleaching events in 2014-2015 were a part of the third and largest global bleaching event to date (Eakin et al. 2016).

Dissertation Objectives

With the rapid acceleration in the presence and severity of bleaching in Hawai‘i, it is important to study the biological phenomenon of coral bleaching to better understand Hawaiian corals' response to these conditions, as well as contribute to the understanding of the effect of thermal stress on the coral holobiont. The chapters of this dissertation explore the acclimatization response of corals to back-to-back thermal stress events, the genomic components of thermal

tolerance in Hawaiian rice coral, *Montipora capitata*, the reproductive and physiological stress of growth anomalies on the Hawaiian lobe coral, *Porites evermanni*, and the effectiveness of citizen science in coral reef monitoring and its implications for education and outreach.

CHAPTER TWO

WHAT DOESN'T KILL YOU MAKES YOU STRONGER: EVIDENCE FOR ACCLIMATIZATION IN CORALS DURING REPEATED NATURAL THERMAL STRESS

Abstract

Understanding how organisms react to changing climate conditions is an important issue for the future of coastal ecosystems worldwide. On coral reefs, bleaching is a stress response to elevated sea water temperatures, the frequency of which is projected to increase as the result of climate change. Corals may adjust their thermal sensitivity through population adaptation or colony acclimatization, but distinguishing between these responses requires long-term ecological observation of individual colonies in the field and a critical question remains: can corals adapt or acclimatize to these changing conditions on relevant timescales? Consecutive bleaching events in the Hawaiian Islands in 2014 and 2015 provided an unprecedented natural experiment – and potential window into future conditions – for comparing bleaching susceptibilities of the local coral community as well as acclimatization responses to annual bleaching events. Individual colonies and surrounding reef areas were monitored for 15 months encompassing time before, during, and after both bleaching events. Metrics of bleaching, recovery, and mortality were calculated using generalized linear mixed models to investigate species-specific responses and recovery trends between bleaching events. I found that *Porites* experienced severe bleaching but recovered with little mortality, *Pocillopora* also experienced high levels of bleaching and mortality but with variable recovery, while *Montipora* experienced variable levels of bleaching with low mortality and moderate recovery. As a whole, the reef experienced less bleaching, greater recovery, but slightly more mortality in 2015 compared to 2014 even though thermal stress exposure was more severe during the 2015 event (11 vs 14 *in situ* degree heating weeks (DHW) in 2014 vs. 2015, respectively). Individually monitored colonies showed lower percentages of total bleached area in 2015, while temperature exposure (DHW) was 27% greater, demonstrating that some corals can acclimate to thermal stress in just one year.

Introduction

A fundamental question for ecologists in the 21st century is how or if organisms can adapt or acclimatize to changing environmental conditions as a result of the effects of global climate change (Hughes et al. 2003; Stillman 2003; Hoffman and Sgro 2011). For reef-building, foundational species like scleractinian corals, the negative effects of climate change are expected to have cascading effects on the function and diversity of the coral reef ecosystem as a whole (Hughes et al. 2007; De'ath et al. 2009; Pandolfi et al. 2011; Hughes et al. 2017, 2018b). Coral populations are threatened by the worldwide phenomenon of coral bleaching (Brown 1997; Hoegh-Guldberg 1999; Hoegh-Guldberg et al. 2007), a stress response induced by elevated sea water temperatures (Coles et al. 1976; Jokiel and Coles 1990) and increased penetration of ultraviolet (UV) or photosynthetically active radiation (PAR) during periods of extended calm conditions (Lesser et al. 1990; Gleason and Wellington 1993). With projected increases in the frequency of thermal stress events (Donner et al. 2005; Hughes et al. 2017; Lough et al. 2018), an important ecological and evolutionary dilemma is whether coral populations can adapt or adjust their sensitivity to climate change over relatively short timescales (van Hooidonk et al. 2013; Hughes et al. 2018a).

While coral populations may adapt in response to elevated stress, the rapid rate of climate change will make acclimatization an important factor in the short-term survival of reefs (Edmunds and Gates 2008), as reefs will likely experience significant bleaching events on a yearly basis by 2050 (Hoegh-Guldberg 1999; Donner et al. 2005; Logan et al. 2013; van Hooidonk et al. 2013; Hughes et al. 2018a). Numerous studies have shown evidence of symbiont community changes after thermal stress (Baker 2001; Baker et al. 2004; Berkelmans and van Oppen 2006; Jones et al. 2008; Baker et al. 2013; Cunning et al. 2015a), as well as host-specific

genomic evidence of coral adaptation to harsh conditions (Smith-Keune and van Oppen 2006; Bellantuono et al. 2012; Lundgren et al. 2013; Palumbi et al. 2014; Dixon et al. 2015). However, the potential for symbiont shifting in corals in response to thermal stress (Buddemeier and Fautin 1993) is not shared universally, sparking a debate about the capabilities of corals to acclimatize to increases in sea water temperatures (Buddemeier and Smith 1999; Hoegh-Guldberg 1999; Kinzie et al. 2001; Coles and Brown 2003; Hughes et al. 2003; Hoegh-Guldberg et al. 2007; Edmunds and Gates 2008). Therefore, despite stress-mitigating mechanisms and some evidence of acclimatization, the potential for individual colonies to acclimatize on timescales relevant to the increasing degree of global climate change remains poorly understood (van Hooidonk et al. 2013).

Studies supporting the ability of corals to acclimate to repeated stress have noted less bleaching in locations that experienced a severe bleaching event in the recent past (Dunne and Brown 2001; Glynn et al. 2001; Jimenez et al. 2001; Podesta and Glynn 2001; Berkelmans et al. 2004; Maynard et al. 2008; Thompson and van Woesik 2009; Guest et al. 2012; Pratchett et al. 2013; McClanahan 2017; Gintert et al. 2018; but see Hughes et al. 2017), however, most long-term reef monitoring used reef-scale survey methods that did not account for the influence of partial colony mortality in inter-annual outcomes. While these studies are consistent with acclimatization with multiple years between bleaching events, it is unknown if reduced bleaching responses were due to population adaptation or individual acclimatization from previous bleaching events, or if they were due to conditioning from fluctuations in local environmental parameters from year to year or changes in community composition and bleaching susceptibility over time (Pratchett et al. 2013; McClanahan 2017). Although some studies monitored the same colonies through time (Dunne and Brown 2001; Glynn et al. 2001; Jimenez et al. 2001; Podesta

and Glynn 2001; Gintert et al. 2018), the difficulty of interpreting reef-scale data, as well as uncertainties in predictions of future ocean stressors, currently limit our understanding of how individual coral colonies will react *in situ* to increasing frequencies of thermal stress. While reef-scale studies allow for greater breadth of monitoring, they cannot account for acute changes in individual colonies' response to stress. Laboratory studies provide controlled environments for well-replicated and isolated testing of these processes, but they cannot determine how individuals will respond in their natural environment. To date, there has been only one definitive study (Gintert et al. 2018) utilizing individual colony level monitoring to observe how corals react to conditions of thermal stress *in situ* during back-to-back thermal stress events.

The Hawaiian archipelago has long been protected from severe worldwide bleaching events (Jokiel and Brown 2004; Rodgers et al. 2015), due to cooler than average sub-tropical sea temperatures (Mantua and Hare 2002). The first two prominent bleaching events in Hawai‘i occurred in the main Hawaiian Islands (MHI) in 1996 and in the Northwestern Hawaiian Islands (NWHI) in 2002 (Jokiel and Brown 2004). However, after an eighteen-year reprieve from severe thermal stress, coral bleaching occurred across the archipelago in both the MHI and the NWHI in 2014 and in the MHI in 2015, resulting in two consecutive bleaching events, a historically unprecedented occurrence for Hawai‘i. In 2014, anomalous atmospheric conditions caused a northward flow that brought extremely warm water to the Hawaiian archipelago, leading to a thermal stress event (Peterson et al. 2015). The second bleaching event occurred as a result of both the 2015-16 El Nino Southern Oscillation (ENSO) and the recent shift from the cool phase to the warm phase of the Pacific Decadal Oscillation (PDO) (Rodgers et al. 2015). The PDO has long influenced ocean temperatures in Hawai‘i (Zhang et al. 1997); the shift to a warm phase combined with the strongest ENSO event on record (Eakin et al. 2016) resulted in anomalously

high ocean temperatures and severe bleaching throughout the MHI in 2015. This study utilized the natural experiment presented by the 2014 and 2015 bleaching events in Hawai‘i to investigate inter- and intra-specific variation in corals response *in situ* to elevated levels of thermal stress on both colony and reef scales. Through colony-level observational monitoring across the back-to-back bleaching events I documented inter- and intraspecific variation in bleaching susceptibility and acclimatization to stressful thermal conditions within a relatively short time span under increasing levels of thermal stress.

Methods

This study was conducted at A‘alapapa Reef (offshore from Lanikai Beach), on the windward side of the island of O‘ahu, Hawai‘i. Seven study sites were established within the lagoon behind the 2.4 kilometer stretch of the reef in June of 2014 (Figure 2.1). Sea water temperature was measured *in situ* from July 2014-December 2015 via HOBO pendant temperature loggers (Onset) at six sites (Figure 2.1), with at least one logger within a maximum of 5 meters of each focal colony. Loggers were wrapped in reflective tape to avoid overheating and subsequent misrepresentation of water temperature (Bahr et al. 2016).

Individual Colony Response

Individual colonies of *Porites evermanni* (26), *Porites lobata* (4), *Pocillopora damicornis* (16), *Pocillopora meandrina* (10), *Montipora capitata* (10), and *Montipora patula* (10) were selected at six sites (Figure 2.1). Corals were monitored weekly from August-December 2014 and bi-monthly from January-December 2015; except for colonies of *Montipora capitata* and *M. patula* that were not included in 2014 and so were only monitored bi-monthly from March-December 2015. These colonies were added in 2015 after predictions for a second bleaching

event were revealed. Relative tissue health of individual colonies were scored as: healthy, bleached, and dead. Additionally, photographs were taken of each colony (to allow for verification of scoring). Each colony was marked with a stake and Coral Watch card (Siebeck et al. 2006) for size and color reference.

Overall Reef Response

At five sites, thirteen 10 m video transects were recorded on nine dates, approximately every two months from September 2014 to December 2015 (Figure 2.1). All transects were perpendicular to shore, separated by approximately 5 meters. Three transects were recorded at each site except for Site 1 due to low coral cover (one transect only). Video was recorded on a Canon G12 camera attached to an extension pole held 40 centimeters above the benthos. Screenshots were taken every 10 seconds and Coral Point Count (CPCe; Kohler and Gill 2006) was used to score the area under 50 points per screenshot (Brown et al. 2004) for benthos type (coral, algae, sand, rock), coral species (*Porites evermanni*, *P. lobata*, *P. compressa*, *Pocillopora damicornis*, *P. meandrina*, *Montipora capitata*, and *M. patula*), and coral health (healthy, pale, mucus sheet covered, bleached, dead). Only points touching coral were utilized for further analysis. Additionally, the only health categories utilized were those representing unhealthy or comprised states (pale, bleached, mucus sheet covered) to determine the impact of the bleaching events on overall reef health. These health categories combined are hereafter referred to as "affected tissue". The values for each health category within a transect for each species were standardized by the total amount of coral scored for all health categories within that transect for each species.

Degree Heating Weeks

To estimate thermal stress, degree heating weeks (^{0}C-weeks) were calculated from *in situ* temperature data using the method of NOAA's Coral Reef Watch Program (Wellington et al. 2001; Liu et al. 2006). Considerable evidence suggests that in many corals, bleaching is likely to occur at approximately 4 ^{0}C-weeks (Liu et al. 2003) and widespread bleaching and coral mortality at approximately 8 ^{0}C-weeks (Liu et al. 2006). *In situ* temperatures were corrected for consistent SST to *in situ* bias using NOAA's 5 km satellite nighttime sea surface temperature product (NOAA Coral Reef Watch, 2013a) by subtracting the mean of the difference between satellite and *in situ* temperatures for each day (*in situ* temperatures recorded every hour previously averaged to produce one value per day). For validation of temperature correction between satellite and *in situ* data see Appendix B: Figure S2.1. Following correction, hotspot values were calculated by subtracting the maximum of the mean monthly temperature from NOAA's Coral Reef Watch 50 km satellite nighttime sea surface temperature climatology product (NOAA Coral Reef Watch, 2000). Hotspot values less than one were equated to zero since NOAA's protocol only sums the values of thermal stress hotspots above one (Liu et al. 2006). NOAA's Coral Reef Watch degree heating week products (NOAA Coral Reef Watch 2013b) are calculated on a twice weekly basis over the period of twelve weeks. Given the difference in timescale of our data points, I produced a degree heating week product using daily hotspot values for a twelve-week period. Daily degree heating week values were divided by 7 to standardize the unit to weeks. Twelve weeks of NOAA's 5 km satellite nighttime sea surface temperature products (NOAA Coral Reef Watch 2013a) were incorporated into the 12-week rolling window for degree heating week calculation. This calculation method presents a mix of *in situ* and satellite data for the first twelve weeks (July 20th- October 11th) of the dataset and likely

led to an under-estimation of peak degree heating weeks for the 2014 bleaching event. Furthermore, *in situ* data represent an important component of the abiotic environment at the time of bleaching, and as such, the data illustrate not only the differences in *in situ* and satellite metrics, but also the specific characteristics at A‘alapapa Reef in 2014 and 2015. For comparison, I have included both *in situ* degree heating weeks and NOAA’s Coral Reef Watch degree heating weeks product (NOAA Coral Reef Watch 2013b) (Figure 2.2). All graphics incorporating degree heating weeks are shown with values for both *in situ* and NOAA’s Coral Reef Watch degree heating week products (Figures 2.2, 2.3, 2.7). Degree heating weeks were compared between years using a sliding window analysis for the weeks where *in situ* degree heating weeks were above 0 ^{0}C-weeks. Each window tested the difference between years using a Wilcox rank-sum test for two-week windows sliding by one week. A sequential Bonferroni adjustment was used to account for multiple tests.

Light

Because bleaching can result from thermal and light stress, photosynthetically active radiation (PAR) values were collected from the MODIS Aqua-Ocean Color data satellite record (NASA 2014; Frouin et al. 1989) at the pixel nearest to A‘alapapa Reef (within ~ 1.5 km). PAR data were compared for the time period when *in situ* degree heating weeks were above 0 ^{0}C-weeks using the sliding window analysis as above.

Statistical Analysis

Colony-level data were split into two 9-month periods, each of which encompassed before, during, and after two consecutive bleaching events. These periods are referred to hereafter as the 2014 event (August 2014-March 2015) and the 2015 event (April 2015-December 2015). To account for pseudoreplication and temporal autocorrelation, the data for

individual colonies was compressed into three metrics for each colony for each event time bracket to examine bleaching, recovery, and mortalityt: 1) a bleaching quotient, the maximum percentage value of bleached tissue over the initial percentage of healthy tissue (B_{max}/H_i) ; 2) a recovery quotient, the final percentage of healthy tissue over the initial percentage of healthy tissue (H_f/H_i) ; and 3) a mortality value, the final percentage of dead tissue minus the initial percentage of dead tissue ($M_f - M_i$).

Generalized linear mixed models were used to examine the effects of species and event (2014 vs. 2015) on bleaching, recovery, and mortality. All metric values above 1.0 represented full colony bleaching, recovery, or mortality and therefore the metric values above 1.0 were set equal to 1.0 so that the data could be modeled using a two-column binomial response. Models were run as bglmer objects from the R package blme (Chung et al. 2013) allowing for setting normal priors on the fixed effects to account for total separation of data (Hauck Donner effect). Additionally, an optimizer was used to account for failures to converge, the R package afex (Singman et al. 2016) was used to test which optimizer would work best for the data and the default optimizer "bobyqa" was appropriate for all proposed models.

Models were run with species and event as fixed effects and colony ID nested within site as random effects. Fixed effects were evaluated by parametric bootstrapping with null models using the R package pbkrtest (Halekoh and Højsgaard, 2014). Multiple comparisons with Tukey adjustments were used to assess inter-specific variation and the differences in bleaching, recovery, and mortality between events using the R package lsmeans (Lenth 2016). All models run on colony level metrics excluded data for the species *Pocillopora damicornis* due to its early bleaching-induced mortality at the beginning of the 2014 bleaching event and the effect these data would have on fixed-effect predictor significance. All graphics presented represent the data

run through each mixed model and therefore do not include *P. damicornis*; see Appendix B: Figures S2.2 – S2.4 for graphics with *P. damicornis* included for comparison.

An additional three models were run with a subset of colonies for all three-health metrics (bleaching, recovery, and mortality); this subset included only colonies that survived throughout the entirety of both bleaching events and excluded the two *Montipora* species (which were only present in the dataset in 2015) and *Pocillopora damicornis* (which all died after the 2014 event). These models were run with the same parameters as above but with an added interaction between the fixed effect of species and event. The interactions were tested using parametric bootstrapping with null models and intra-specific variation of each species bleaching, recovery, and mortality responses between bleaching events was tested using multiple comparisons with multivariate (mvt) adjustments.

A similar mixed model was used to investigate differences between species and date for the video transect data. Affected tissue was the response variable with species and date of video recording as fixed effects and transect ID nested within site as random effects. Fixed effects were evaluated with parametric bootstrapping with null models and inter-specific and inter-annual variation was evaluated with multiple comparisons with Tukey adjustments. Like the colony level data, a second model was run on the video transect data using only the data from September and October of both years. These months experienced the highest bleaching response in 2014 and therefore transects were recorded for both months in 2015 for comparisons. This model used affected tissue for the months of September and October as the response variable and species and year as fixed effects with an interaction and transect ID nested within site as random effects. The interaction was tested with parametric bootstrapping with null models and the intra-specific and

inter-annual variation was tested using multiple comparisons with a multivariate adjustment. All analyses and graphics were completed using R 3.3.0 (R Core Team 2016).

Results

Degree Heating Weeks

In both years, *in situ* temperatures peaked at 30.9 ^{0}C, although temperatures peaked on different dates: September 21st in 2014 and August 29th in 2015 (Figure 2.2). NOAA Satellite SSTs peaked on the same day as *in situ* temperatures in 2014 at 28.3 ^{0}C and on September 6th in 2015 at 29.0 ^{0}C (Figure 2.2). While peak sea temperatures were similar both years, summed thermal stress was greater in 2015 than in 2014. The degree heating weeks calculated using *in situ* data indicated that A'alapapa Reef experienced 11 ^{0}C-weeks in 2014 (NOAA Coral Reef Watch max = 2.1 ^{0}C-weeks) and a 14 ^{0}C-weeks in 2015 (NOAA Coral Reef Watch max = 7.3 ^{0}C-weeks) (Figure 2.2). The sliding window analysis revealed that all but two of the twenty-eight windows from June 7th-December 14th showed a significant difference in degree heating weeks between years. The windows without significant differences occurred from October 4th–October 17th, where degree heating weeks were high both years and December 13th-December 14th, where degree heating weeks were low or zero both years. In windows before October 11th, degree heating weeks were greater in 2015 than 2014, and in windows after October 11th, degree heating weeks were greater in 2014 (Figure 2.2).

Light

Maximum PAR was 1400 μmol/m^{2}s, which occurred on July 6th, 2014 and 1398 μmol/m^{2}s on July 9th, 2015. Maximum PAR values when *in situ* degree heating weeks were greater than zero occurred on August 6th in 2014 and August 9th in 2015, and were the same as

the overall maximum PAR values for 2014 and 2015. The sliding window analysis for this time period showed only one window with significantly different levels of PAR between years (p-value = 0.0001). During this window (September 13th – September 26th) mean PAR was greater in 2014 than in 2015 by 209.67 μmol/m^{2}s (17%). Additionally, the difference in mean PAR between years for the entirety of the window in which thermal stress was present (*in situ* degree heating weeks > 0) was 39 μmol/m^{2}s (Wilcox rank sum: p = 0.303), a difference of 3% less PAR in 2015 compared to 2014 (Figure 2.3).

Individual Colony Response

Analysis of colony level responses revealed significant differences in bleaching, recovery, and partial mortality both among species and bleaching events.

Bleaching: Parametric bootstrapping of colony level bleaching quotients (B_{max}/H_i) mixed model fixed effects showed a significant species effect (p = 0.002) regardless of event and a significant effect of event overall (p = 0.001) between the two years regardless of species. Multiple comparisons of species effects revealed significantly less bleaching in *M. patula* compared to each of *P. evermanni* (p < 0.001) and *P. meandrina* (p = 0.002).

Recovery: Parametric bootstrapping of colony level recovery quotients (H_f/H_i) mixed model fixed effects showed a significant effect (p = 0.001) of species recovery patterns regardless of event and a significant effect (p = 0.001) of event regardless of species. Multiple comparisons of species effects revealed significantly less recovery of *P. lobata* in comparison to each of *P. evermanni* (p = 0.003), *M. capitata* (p = 0.001), and *M. patula* (p = 0.001), and significantly less recovery in *P. meandrina* and each of *P. evermanni* (p < 0.001), *M. capitata* (p < 0.001), and *M. patula* (p < 0.001).

Partial Mortality: Parametric bootstrapping of colony level mortality values ($M_f - M_i$) mixed model fixed effects showed a significant effect ($p = 0.001$) of species mortality susceptibilities regardless of event, and a significant difference ($p = 0.03$) between events regardless of species for mortality susceptibilities. Multiple comparisons of species effects revealed significantly less mortality in *P. evermanni* than *P. lobata* ($p = 0.022$), *P. meandrina* ($p = 0.003$) and *M. capitata* ($p = 0.015$), and more mortality in *P. evermanni* than *M. patula* ($p = 0.006$). Comparisons also revealed significantly more mortality in *P. meandrina* than in *M. capitata* ($p = 0.005$) and *M. patula* ($p = 0.001$). Additionally, all 16 colonies of *P. damicornis* underwent complete mortality during the 2014 bleaching event and 3 colonies of *P. meandrina* experienced complete mortality either before or during the 2015 bleaching event. (See Appendix A: Tables S2.1 - S2.3 and Appendix B: Figure S2.2a-f for individual species and event bleaching, recovery, and mortality response graphics and p-values.)

In addition to significant differences among colony responses by species and event, I also found significant differences in how the species that survived both events reacted, i.e. significant species by event interactions. Parametric bootstrapping for the fixed effect of the interaction between species and event revealed significant differences for the bleaching model ($p = 0.001$; Figure 2.4a), recovery model ($p = 0.001$; Figure 2.4b), and the partial mortality model ($p = 0.001$; Figure 2.4c). Multiple comparisons of the interactive fixed effects showed significantly less bleaching in 2015 compared to 2014 for *P. evermanni* ($p < 0.0001$), *P. lobata* ($p < 0.0001$), and *P. meandrina* ($p < 0.0001$) (Figure 2.4a). Multiple comparisons of the interactive fixed effects showed significantly less recovery in 2015 compared to 2014 for *P. evermanni* ($p < 0.0001$) and *P. meandrina* ($p < 0.0001$), but there was no significant difference in recovery response for *P. lobata* ($p = 0.071$) between the two events (Figure 2.4b). Multiple comparisons

of the interactive fixed effects showed significantly more mortality in 2015 compared to 2014 for *P. evermanni* ($p < 0.0001$) and *P. meandrina* ($p = 0.001$), and significantly less mortality in 2015 compared to 2014 for *P. lobata* ($p < 0.0001$) (Figure 2.4c).

Overall Reef Response

The reef-scale video transects also showed clear distinctions among species sensitivities and recording dates. Parametric bootstrapping of fixed effects from mixed models run on affected tissue from video transects revealed significant differences between species regardless of the date of the transect ($p < 0.001$) (see Appendix B: Figure S2.3) and date regardless of the species ($p < 0.001$) (Figure 2.5). Multiple comparisons of species effects showed significant differences in affected tissue (bleaching, paling, or mucus sheet covered) between all combinations of species with *M. capitata* > *P. evermanni* > *P. compressa* > *M. patula* > *P. lobata* > *P. meandrina*. Multiple comparisons of the effect of date irrespective of species interactions on tissue affected showed several significant interactions between peak bleaching months for both years (Figure 2.5). The extent of affected coral tissue in video transects peaked in September in both years, with significantly higher affected tissue than in October (2014: $p < 0.0001$; 2015: $p < 0.0001$). Comparing across events, however, extent of affected tissue in September 2014 was not significantly different from those of transects recorded in September 2015 ($p = 0.343$), but extent of affected tissue in October 2014 was significantly higher than those in October 2015 ($p < 0.0001$). (See Appendix A: Tables S2.4 - S2.5 for species and date comparison p-values.)

Comparisons of the interaction of species and date for peak bleaching months (September/October) each year revealed differences in the amount of affected tissue between species and years, and all species except *P. meandrina* experienced less impact during peak

bleaching months in 2015 compared to peak bleaching months in 2014 (Figue 2.6). Parametric bootstrapping of the mixed model run on affected tissue from September and October video transects for both years was significant for the fixed effect interaction of species and event ($p < 0.001$). Multiple comparisons of the interaction between species and event indicated significant differences in species response between each bleaching event for *P. evermanni* ($p < 0.0001$), *P. meandrina* ($p = 0.003$), and *M. patula* ($p < 0.0001$) (Figure 2.6). No significant differences were found between the level of affected tissue from 2014 to 2015 for *P. compressa*, *P. lobata*, or *M. capitata* (Appendix A: Table S2.6). A multiple comparison of the fixed effect of event (without an interaction between species) revealed significant differences with more affected tissue in September and October 2014 than in September and October 2015 ($p < 0.001$).

Discussion

In back-to-back thermal stress events in Hawai‘i, individual coral colonies bleached less during the second year, even though peak SST was nearly identical between events and accumulated thermal stress was far greater in the second event. The use of colony-level monitoring allowed us to make observations of bleaching responses, recovery, and tissue mortality of individual colonies over the course of two bleaching events without the confounding impact of unknown partial mortality that would misrepresent reef-scale inferences about acclimatization. The patterns of impact of back-to-back events showed that, in 2015, colonies bleached significantly less (regardless of species), recovery was similar, and mortality was slightly higher than in 2014. The lower bleaching response of corals in 2015 was surprising given that, even though the maximum temperatures were similar between years, the cumulative buildup of thermal stress was greater in the second event.

The difference in bleaching responses between years is evident in the summary of the bleaching quotient for each individual versus the degree heating weeks (^{0}C-weeks) on the day each colony experienced its maximum bleaching (Figure 2.7). In 2015, for all three species, maximum bleaching quotients were lower than the values in 2014 for each species and for the year as a whole, even though the number of degree heating weeks experienced in 2015 was higher than these corals experienced in 2014 (Figure 2.7). This shows acclimatization of these colonies in a short time period to the effects of thermal stress. A similar result was found for coral colonies in the Florida Keys during the 2014 and 2015 thermal stress events (Gintert et al. 2018). When using photomosaic quadrats to monitor individual colonies across both events, they found less bleaching and less mortality in colonies across a number of major Caribbean reef species the second year despite greater thermal stress. These results provide further evidence that coral colonies can acclimatize in the field under the timescale of back-to-back annual bleaching events.

These trends were consistent for all three species (*P. evermanni*, *P. lobata*, and *P. meandrina*) monitored over two years, even though these species span the spectrum for typical bleaching susceptibilities (Marshall and Baird 2000; Loya et al. 2001; Wooldridge 2014a). There was less bleaching and recovery was the same or slightly lower in 2015, possibly due to a lack of capturing complete recovery since monitoring ceased in December 2015. Mortality increased slightly in 2015 except for *P. lobata*, which experienced lower partial mortality in the second bleaching event. This may have been due to the large amount of partial mortality experienced by colonies in 2014 and the subsequent decrease in living tissue present in 2015. While massive *Porites* species are generally regarded as more tolerant and hardy species (Loya et al. 2001), some reports of high mortality during *in situ* thermal stress events have been reported for *P.*

lobata in French Polynesia (Mumby et al. 2001) and *Porites* sp. in South East Asia (Guest et al. 2012). Therefore, while surprising, this increased bleaching susceptibility in *P. lobata* was not an anomalous observation.

Overall reef response was similar; for five of the six species followed on video transects, the amount of affected tissue in September and October was less in 2015 than 2014 (Figure 2.6). This overall reef response aligned with the response of individual colonies (Figure 2.7), which is consistent with the hypothesis that acclimation occurred. A number of studies (Brown et al. 2002a, 2002b; Castillo and Helmuth 2005; Ulstrup et al. 2006; Middlebrook et al. 2008; Thompson and van Woesik 2009; Guest et al. 2012) have shown that the thermal history of a location influences the overall susceptibility of a reef to future exposure of thermal stress. While these studies have demonstrated this concept on a timescale relevant to multiple years between stress events, I demonstrate that survival from exposure to extreme thermal stress lowered the susceptibility to bleaching of the same colonies and reef scape to the next, similar stress event, only twelve months later. And these responses represent novel inferences on reef bleaching in annual thermal stress events and provide a more accurate comparison by monitoring reef dynamics at the colony level.

However, corals' responses to stress are influenced by a number of environmental variables that, along with differences in pre-stress conditions, could influence the magnitude of stress response. Differences in pre-stress thermal fluctuations may explain Kenyan corals' response to thermal stress periods in 1998 and 2016 (McClanahan 2017), where they found that pre-stress spikes in temperature may have pre-conditioned corals to bleaching stress. The reefs experienced the same levels of maximum thermal stress in 1998 and 2016, but the corals showed different levels of bleaching severity between years. In our study, Hawaiian corals in 2014 were

exposed to high levels of thermal stress with little pre-stress fluctuation in normal summer peak temperatures. In 2015, *in situ* SST spiked at the beginning of July, then cooled off slightly before a stretch of sustained thermal stress in late August. The 2015 spike in temperature, rather than the 2014 bleaching event, may have pre-conditioned corals to better withstand the coming thermal stress (Ainsworth et al. 2016). However, the pre-stress spike was of the same magnitude in temperature as the later sustained thermal stress, both above the local bleaching threshold. This differs from the protective trajectory temperature pattern where the pre-stress spike rises above mean monthly maximum temperatures but remains below bleaching threshold temperatures (Ainsworth et al. 2016). Therefore, it is more likely that the Hawaiian corals had acclimatized from the 2014 event.

PAR is also associated with the stress response of bleaching (Lesser et al. 1990; Marshall and Baird 2000). I found slight PAR differences between years (3% less PAR in 2015 when DHW > 0) that point to less light in 2015 compared to 2014. However, PAR was 17% greater for one window of time in 2014 (September 13th to September 26th). While bleaching was occurring during this two-week time period in both years, the decreased level of PAR may have lessened the stress on corals in 2015. The large difference in *in situ* temperature stress between years during those two weeks (mean: 2014- 6.1 ^{0}C-weeks, 2015- 13.7 ^{0}C-weeks) was likely a more important source of stress on the corals than differences in PAR (Figure 2.3).

There was notable interspecific and inter-colony variation in biotic data within and between the two bleaching events at A‘alapapa Reef. This is not surprising given that previous studies have shown how physical and physiological traits of the coral host can influence bleaching susceptibility (Gates and Edmunds 1999; Loya et al. 2001; Baird et al. 2008; Fitt et al. 2009; Edmunds et al. 2014). At the colony level, *Porites* and *Pocillopora* were both susceptible

to bleaching while *Montipora* (encrusting and branching growth forms) was less susceptible but with greater inter-colony variability. *Montipora capitata* in Hawai‘i have been found to utilize heterotrophic feeding more than *Porites* species during thermal stress (Grottoli et al. 2006). As such, this method of nutrient intake may have assisted with the decreased bleaching susceptibility and intra-colony bleaching variability observed in *Montipora*.

The two massive *Porites* species, *P. evermanni* and *P. lobata,* differed unexpectedly in recovery and mortality trends. The *P. lobata* colonies bleached to a bright yellow instead of white, likely due to fluorescence from host pigments, yet these individuals suffered high mortality compared to the *P. evermanni* colonies, which lost their purple or brown pigments completely (to the naked eye) and recovered with little to no mortality. Given that host fluorescent pigments are known to provide photo-protection (Salih et al. 2000, 2006) and thus confer some level of bleaching resistance, it is surprising that *P. lobata* experienced greater mortality than *P. evermanni*. Perhaps other environmental factors associated with colony micro-environments, or variable molecular or physiological holobiont traits played a role in influencing the differential response between these two species, such as the increased rate of mucus sheet production on *P. evermanni* versus *P. lobata*. Soon after the onset of thermal stress in late August and early September, colonies of *P. evermanni*, already pale and partially bleached, became thickly covered in mucus sheets and when the sheets sloughed off weeks later, the tissue underneath was fully recovered, while the rest of the *Porites* colonies were still only beginning to recover. Previous work has identified mucus as a protective mechanism against invasion by microbes (Brown and Bythell 2005; Ritchie 2006), and perhaps the mucus sheets also provide a refuge from light for corals following bleaching events.

Overall reef transect data showed that *Porites* was moderately susceptible to bleaching with the highest declines in health seen in *P. evermanni*; *M. capitata* showed greater susceptibility to bleaching and *P. meandrina* showed lower susceptibility than in the individual colony data. This observed trend in *P. meandrina* was likely due to its low abundance in the video transects as *Pocillopora* are generally considered a stress sensitive species (Loya et al. 2001). There are many colonies of *Montipora* at A‘alapapa Reef, particularly *M. capitata*, so it is possible that the 10 colonies followed over time did not adequately represent the trends of the whole population as the transects picked up a greater reef-scale bleaching signature.

Like the individual colony bleaching trends, recovery as assessed in video transects was high in *Porites* (aside from *P. lobata*) and *Montipora* and lower and more variable in *Pocillopora*, which was expected from taxa regarded as more tolerant (*Porites* and *Montipora*) and the taxon regarded as more susceptible (*Pocillopora*) (Wooldridge et al. 2014a). Following the established susceptibility trend, *Porites* (except *P. lobata*) and *Montipora* exhibited the least mortality and *Pocillopora* showed the most mortality.

Both the individual colony-scale and the reef-scale responses of Hawaiian corals showed that acclimatization can occur *in situ* on short timescales where bleaching stress is annual. With the projected rise in greenhouse gas emissions (IPCC 2014) and subsequent warming of the oceans, corals will have to continue to keep up with the increasing level of thermal impacts. While several studies have already begun to broaden our understanding of this process (Logan et al. 2013), more information will be needed to understand the physiological and genetic mechanisms driving acclimatization and how these mechanisms vary by species and geographic location (Bay et al. 2017). Corals will have to not only respond and acclimatize to thermal stress, but to additional local stressors (e.g. sedimentation, overharvest of herbivores) as well (Donner et

al. 2005; Hoegh-Guldberg et al. 2007). Without the reduction of greenhouse gas emissions and mitigation of local stressors, the fate of coral reefs, some of the most productive and important ecosystems on this planet, will rest in the physiological and evolutionary capabilities of the organisms that form the building blocks of that ecosystem.

Acknowledgments

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Figures

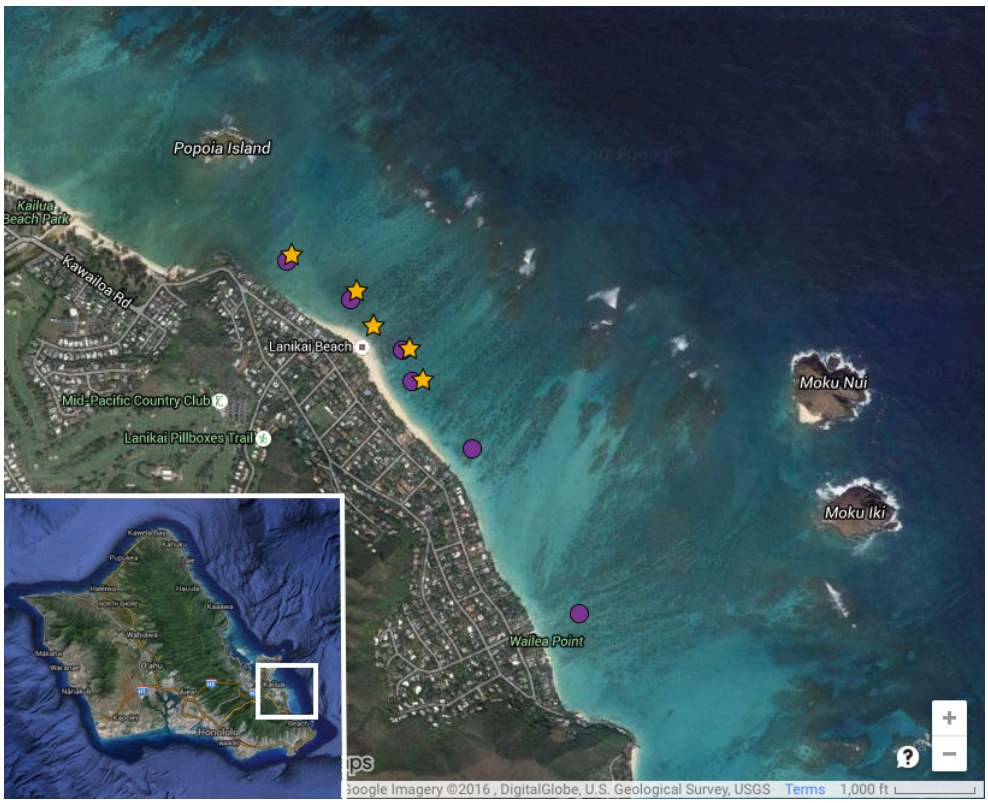

Figure 2.1: Monitoring sites (individual colonies followed at dots and video transects recorded at stars) along the shallow A‘alapapa reef flat off Lanikai Beach, O‘ahu, Hawai‘i.

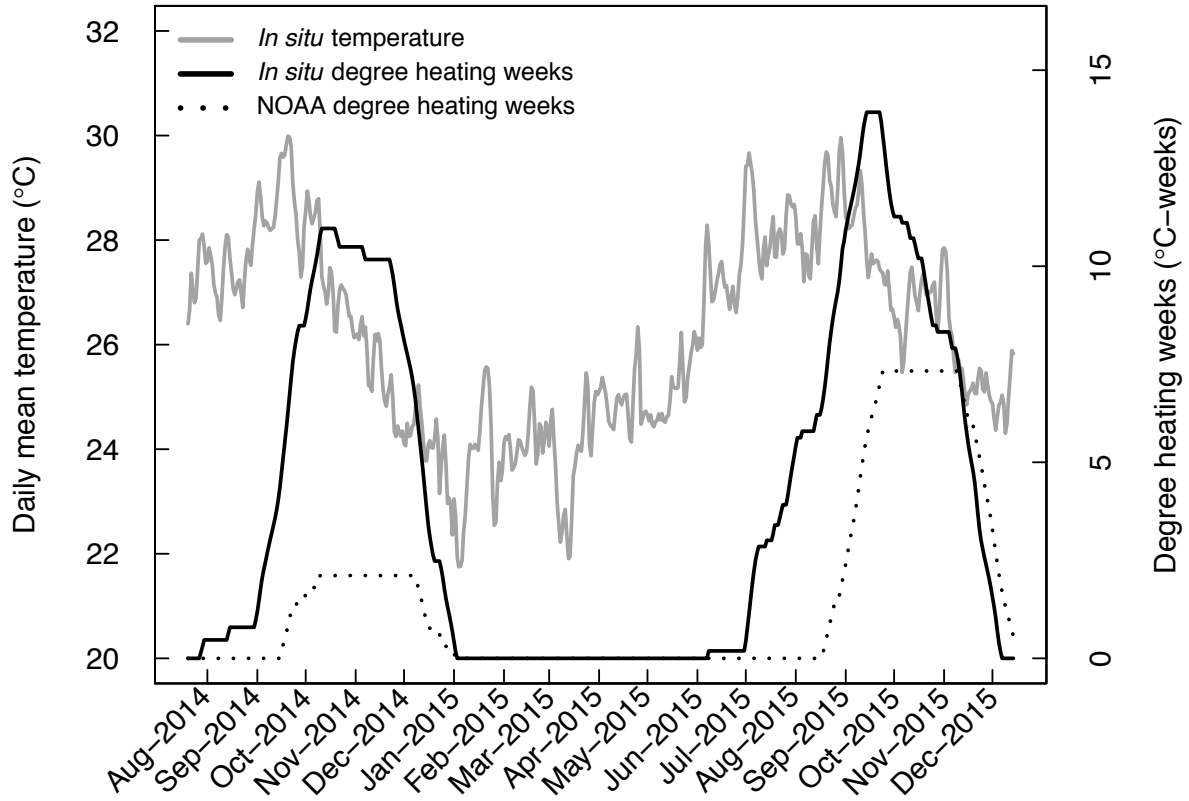

Figure 2.2: Corrected temperature (^{0}C) and degree heating weeks (^{0}C-weeks) calculated using *in situ* daily hotspot values and NOAA degree heating weeks (^{0}C-weeks) for A‘alapapa Reef from July 20, 2014-December 15, 2015.

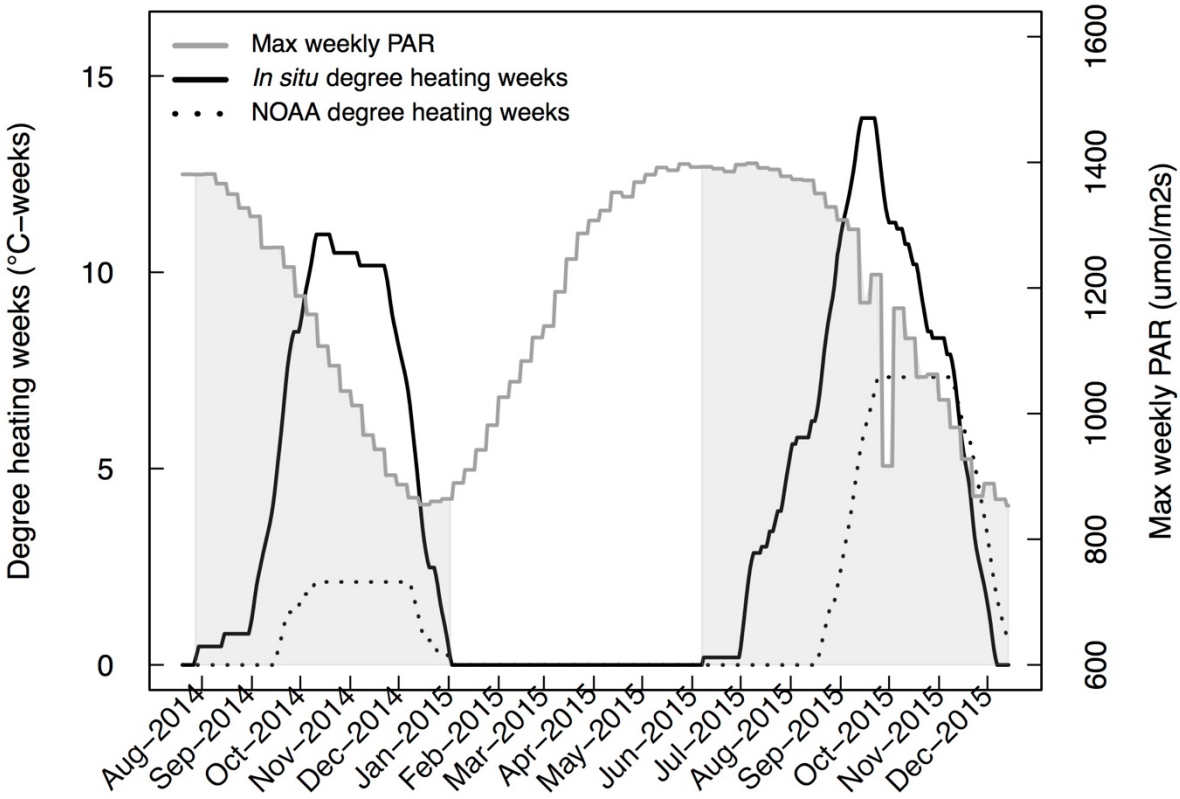

Figure 2.3: Weekly maximum of daily PAR values (umol/m^2s) and degree heating weeks (^{0}C-weeks) calculated using *in situ* daily hotspot values and NOAA degree heating weeks (^{0}C-weeks) for A‘alapapa Reef from July 20, 2014-December 15, 2015. Area shaded denotes PAR exposure during time period where *in situ* degree heating weeks > 0.

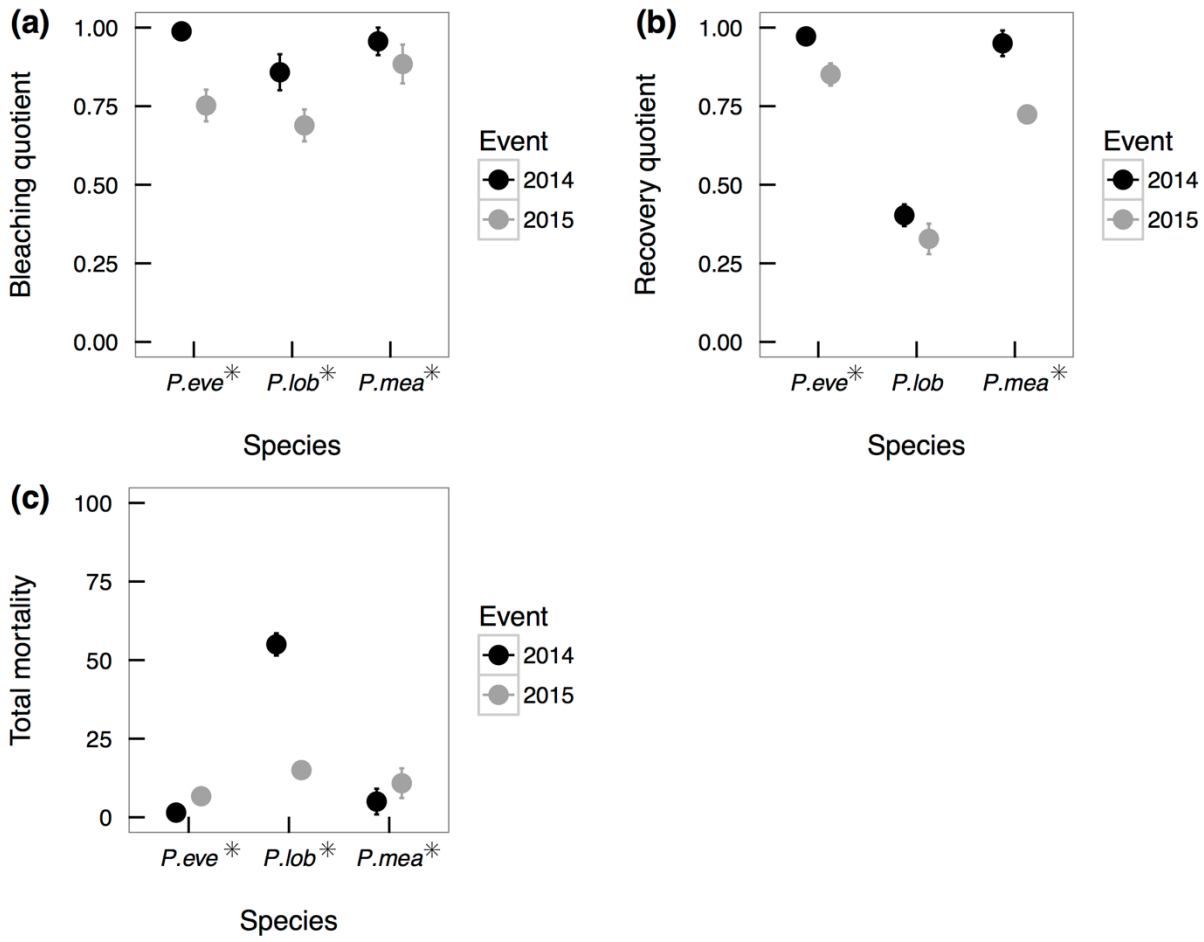

Figure 2.4: (a) Mean bleaching quotients ($B_{max}/H_i \pm SE$) (b) mean recovery quotients ($H_f/H_i \pm SE$), and (c) mean mortality values ($M_f - M_i \pm SE$) for *Porites evermanni*, *Porites lobata*, and *Pocillopora meandrina* for the 2014 and 2015 bleaching events (significant comparisons between years denoted with stars).

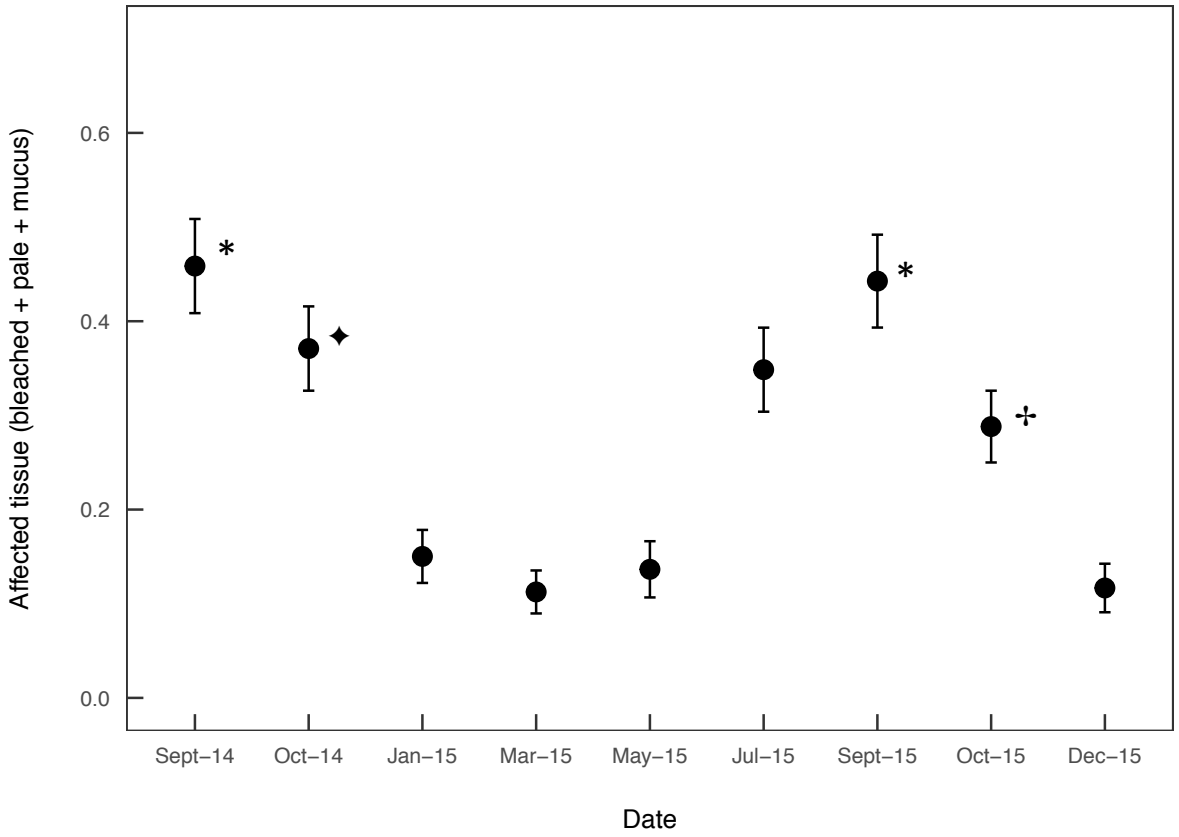

Figure 2.5: Mean affected tissue (bleached + pale + mucus sheets) (± SE) as assessed on each video transect date for all species combined from September 2014 to December 2015 (significant comparisons between peak bleaching months denoted with symbols).

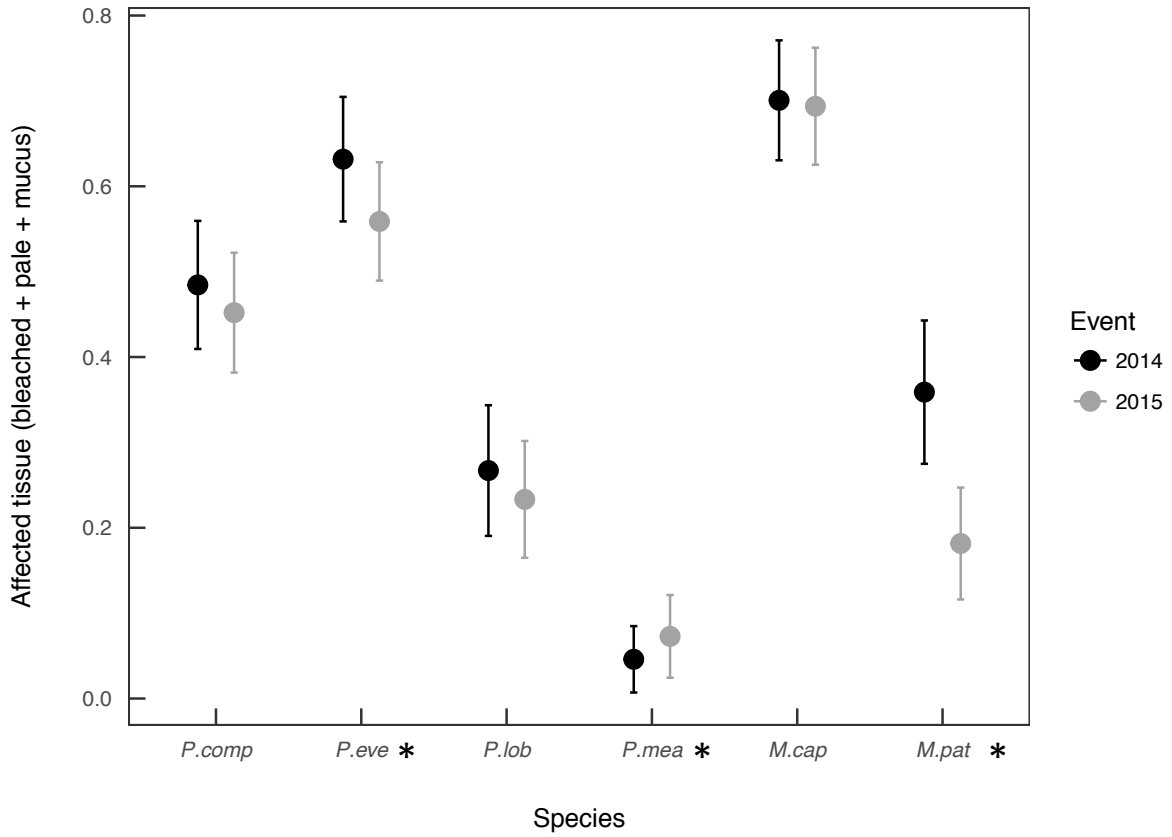

Figure 2.6: Comparison of mean affected tissue (bleached + pale + mucus sheets) (± SE) for *Porites compressa*, *Porites evermanni*, *Porites lobata*, *Pocillopora meandrina*, *Montipora capitata*, and *Montipora patula* in September and October of 2014 and 2015 (significant comparisons denoted with stars).

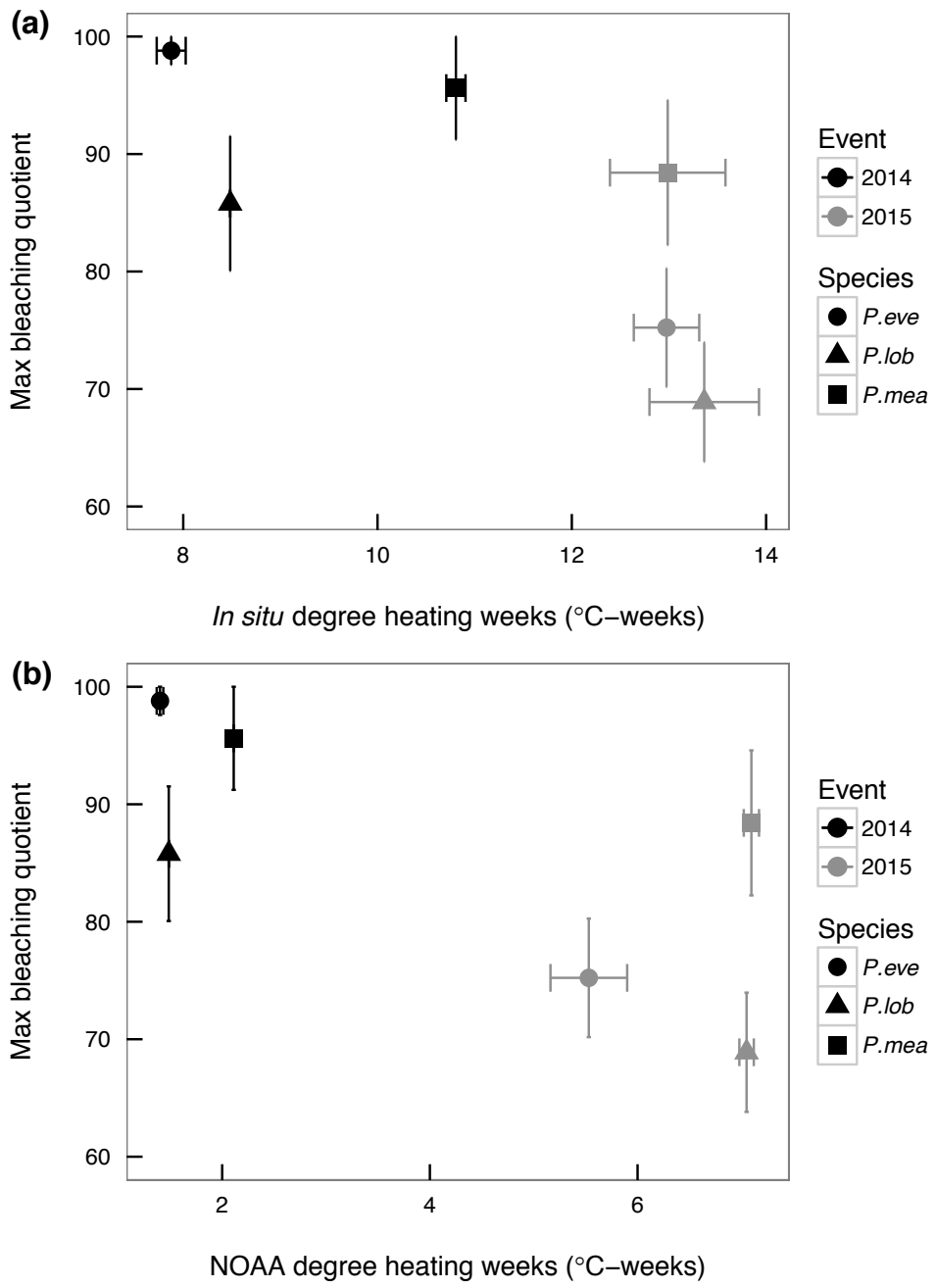

Figure 2.7: Mean maximum bleaching quotients ($B_{max}/H_i \pm SE$) versus (a) mean *in situ* degree heating weeks (^{0}C-weeks ± SE) and (b) mean NOAA degree heating weeks (^{0}C-weeks ± SE) on days of max bleaching for *Porites evermanni*, *Porites lobata*, and *Pocillopora meandrina* in 2014 and 2015.

CHAPTER THREE

GENOMIC ANALYSIS OF DIFFERENTIAL BLEACHING RESPONSES TO IDENTICAL THERMAL STRESS IN *MONTIPORA CAPITATA* IN HAWAI‘I

Abstract

One of the most prevalent threats to marine ecosystems is climate change. Corals, which are the foundation of reef ecosystems, are thermally sensitive species that live at the upper limits of their thermal threshold. Corals' adapt to increasing frequency of thermal stress by fixation of beneficial alleles from standing genetic variation or by the accumulation of *de novo* mutations. The adaptive capacity of coral to thermal stress has been studied extensively through genotype by environment association studies, but few studies exist of genotype by phenotype associations. The 2014 bleaching event in Hawai'i presented an opportunity to study *in situ* the underlying genomic variation behind variable phenotypic thermal stress responses of individuals located side by side. RADseq techniques were used to scan the holobiont genomes of bleached and non-bleached *Montipora capitata* for underlying signatures of selection that might explain differential bleaching responses. Outlier analysis found no signatures of directive selection in the genome, and no significant differentiation and genetic variability were found through pairwise F_{st} Fischer's exact tests and heterozygosity tests between phenotype groups. The lack of genetic differences associated with intraspecific bleaching suggests that variability in bleaching responses are largely phenotypic or explained by factors other than host genotype. In contrast to the lack of differences found in genetic makeup, STRUCTURE analysis and Discriminant Analysis of Principal Components identified some population structure in windward O'ahu sites. This structure may have clouded signals of selection or the differential bleaching responses represented acclimation via phenotypic plasticity rather than adaptive responses.

Introduction

Determining whether adaptation and acclimatization in corals will sufficiently counteract anthropogenically generated thermal stress is an increasingly important area of study within the field of evolutionary biology as evidence increasingly shows the impacts of climate change are rapidly accumulating in every major marine ecosystem (Holderegger et al. 2008; Savolainen et al. 2008; Merila and Hendry 2014; Bay et al. 2017). High sea surface temperatures and high ultraviolet light result in the physiological stress response-coral bleaching, caused by a breakdown of the symbiosis between the coral host and symbiotic dinoflagellate algae (Coles and Brown 2003). Bleaching can also lead to cellular damage and expulsion of algal symbionts, reducing fitness and resulting in partial or complete colony mortality (Weis 2008).

The responses of corals depend on their ability to survive during periods of rapid increase in ocean temperatures by acclimatizing, genetic adaptation, or shifting their spatial distributions (Palumbi et al. 2014; Dixon et al. 2015; Kleypas et al. 2016). Two important major ways in which acclimatization occurs are by a change in coral host physiology and algal symbiont type. Additionally, corals can alter their patterns of expression of heat shock proteins, antioxidant enzymes, apoptosis regulation, innate immune response, and other thermal tolerance related genes (which is often influenced by their thermal history) (Bellantuono et al. 2012; Barshis et al. 2013; Bay et al. 2013; Kenkel et al. 2013a; Palumbi et al. 2014; Kenkel and Matz 2016; Lee et al. 2018; Thomas et al. 2018). Variation in colony-level bleaching response to the same environmental stress conditions has been noted in the field and in controlled experiments (Marshall and Baird 2000; Nakamura and van Woesik 2001; Brown et al. 2002a, 2002b; Cunning et al. 2016; Guest et al. 2016; Tilstra et al. 2017). The variable responses are often attributed to “shuffling” of symbionts, with shifts to a more thermally-tolerant symbiont type

(Baker et al. 2004; Rowan 2004; Berkelmans and van Oppen 2006; Jones et al. 2008). However, other studies have shown the opposite, identifying instances where symbionts did not differ between individuals despite exhibiting differing bleaching responses (Goulet 2006; Kenkel et al. 2013b; Cunning et al. 2016).

In contrast to acclimatization, adaptation occurs across generations within a population either through the successive fixation of beneficial *de novo* mutations or more rapidly by selection on standing genetic variation (Barrett and Schluter 2008; Savolainen et al. 2013; Matuszewski et al. 2015). Given the rapid pace of climate change, an important question is whether coral holobiont populations contain enough standing genetic variation to allow a rapid increase in fitness among individuals of these populations (Donner et al. 2009; Kelly et al. 2012; Logan et al. 2013; Bay et al. 2017). Limited evidence for adaptation of the coral host genome has been found through studies of populations along environmental gradients (Smith-Keune and van Oppen 2006; Bay and Palumbi 2014; Dixon et al. 2015; Kenkel et al. 2015b). For example, Bay and Palumbi (2014) identified a number of candidate loci associated with increased thermal tolerance in corals in American Samoa, and proposed that these populations harbor a pool of thermally tolerant alleles that may allow them to survive future warmer ocean temperatures.

There are two approaches commonly used to search for adaptive potential within populations (Merila and Hendry 2014; Tiffin and Ross-Ibarra 2014). One method used commonly in coral biology is to look for genotype by environment association using existing environmental gradients and reciprocal transplant experiments (Thomas et al. 2018). The other method is to attempt to estimate the level of standing genetic variation within a population by measuring genotype by phenotype associations. While the latter approach has been used in only a few studies, single nucleotide polymorphisms (SNPs) provide an ideal method for studying the

association between genotypes and phenotypes. SNPs are the most abundant type of genetic marker and are typically found at high densities making them especially well-suited for genome wide scans (Baird et al. 2008; Garvin et al. 2010; Bay and Palumbi 2014). In this study, I used RADseq (restriction site associated DNA sequencing) methods to scan the holobiont genomes of Hawaiian *Montipora capitata* to identify SNPs. These were then used to investigate differential phenotypic responses under uniform environmental conditions and levels of thermal stress to answer the following questions: *are there genotypic differences associated with phenotypic variation in bleaching susceptibility of colonies living in the same location on a reef, and do these differences provide insight into their adaptive capacity through standing genetic variation, which may promote survival of future populations in a changing environment?*

Methods

Sample Collection

Study sites were located on the windward side of Oʻahu in Kaneʻohe Bay, at Lanikai Beach, and at Kaiona Beach (Waimānalo) (Figure 3.1). Kāneʻohe Bay consists of several shallow patch reefs and an outer barrier reef, while Lanikai Beach and Kaiona Beach are characterized by shallow reef lagoons and outer fore reefs. Twenty samples (10 bleached and 10 unbleached) of *M. capitata* were collected where colonies exhibiting opposite bleaching responses existed in close proximity to each other (within ~3 m) from each of the study sites in September of 2014 during a mass bleaching event (Eakin et al. 2016). Samples from Kāneʻohe Bay were collected from the shallow reef surrounding Moku O Loʻe where the Hawaiʻi Institute of Marine Biology is located. Samples from Lanikai and Waimānalo were collected from

shallow reef lagoon habitat. Samples were flash frozen in liquid nitrogen and stored at -80 °C until analysis.

DNA Extraction

Whole coral samples (animal tissue, symbionts, and skeleton) were crushed and DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen) using the protocol developed by Toonen et al. (2013). DNA was eluted in HPLC grade water instead of the provided buffer so sample volume could be reduced in a SpeedVac to concentrate the DNA without the worry of concentrated buffer salts for downstream steps. Extractions were examined on a 1.3% agarose gel with TBE buffer and GelRed (Biotum Inc.). Samples with small fragments were subsequently cleaned with Agencourt AMPure XP beads (Agencourt Bioscience Coorperation, Beckman Coulter) at a ratio of 1:0.5 DNA to beads. Following the bead clean, DNA concentration was quantified for each extraction using a Qubit 3.0 fluorometer (Life Technologies, Thermo Fischer Scientific) and the Qubit dsDNA HS Assay Kit (Invitrogen, Thermo Fischer Scientific). Samples containing a minimum of 300 ng DNA/25 μl proceeded through library prep.

Symbiont genotyping

To identify symbiont types within each sample, every individual was genotyped with internal transcribed spacer (ITS) primers and at least one other gene (either psbA or cp23) to verify the dominant clade of *Symbiodinium* for that colony. ITS primers ITSDINO-F (Pochon et al. 2001) and ITS2REV2 (Stat et al., 2009), along with cp23 primers 23HYPERUP and 23HYPERDN (Santos et al. 2003) and psbA primers (LaJeunesse and Thornhill 2011) were used to genotype each individual. The psbA primers were modified so that they amplified a shorter

sequence and were not clade specific (5' TGGATGGGWAGAGAATGGGA and 3' ARCCATGAGCWGCTGAWATRCT).

Amplifications were conducted in 13 µL volumes consisting of 6.3 µL MyTaq 2x (Bioline, USA), 0.3 µL of forward and reverse primers, 0.65 µL BSA, 4.65 µL Ultrapure distilled water (Life Technologies), and 0.5 µL DNA. Thermal cycling parameters consisted of an initial denaturation step of five minutes at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at the annealing temperature, and 45 s at 72 °C, with a final extension of 10 minutes at 72 °C. Libraries were prepared for sequencing with Nextera XT (Illumina, USA) index adapters followed by purification with Agencourt© Ampure XP beads. Amplicons (including negative controls) were then (pair-end) sequenced on an Illumina MiSeq platform (300 bp reads) at the Advanced Studies in Genomics and Proteomics lab at the University of Hawai'i at Mānoa.

The analysis of amplicons from metabarcoding analyses was a two-step process. First, unique sequences or operational taxonomic units (OTUs) were identified using all the sequence reads from every amplicon generated. Second, reads from individual amplicons were re-assembled to OTUs and the number of sequence reads of each OTU were counted for each individual sample. The first step involved the pairing of reads, trimming, and quality filtering (Q=20) with Geneious© 10.0.7. Reads from the entire dataset were pooled and singletons and chimeras were identified and removed prior to clustering with USEARCH v9.2.64 (Edgar 2010). OTUs were compared with Blastn to the NCBI nucleotide database with a e-value cutoff = e^{-30}. Lastly, OTU tables were generated by assembling and tabulating reads of individual amplicon libraries with USEARCH. The number of sequence reads that were present in negative controls was subtracted from the sequence abundances in the corresponding field samples (Nguyen et al.

2015). Thermally tolerant clade D *Symbiodinium* (Stat and Gates 2011) was the major symbiont in one library from Kāneʻohe Bay. This sample was excluded from all subsequent analysis.

Library Preparation

Genomic DNA was digested using the isoschizomer restriction enzymes DpNII and MboI (New England Biolab, Inc), which both cleave at GATC recognition sites. Digestions were run in 50 µl reactions consisting of 25 µl DNA, 19 µl HPLC H_2O, 5 µl buffer, and 1 µl DpNII or MboI with the following thermocycler profile: 37 °C for 3 hours followed by 65 °C for 20 minutes. Following digestion, genomic DNA was cleaned using Agencourt AMPure XP beads at a ratio of 1:0.8 (DNA:beads). Following the post-digestion bead clean, DNA was quantified using the Qubit 3.0 fluorometer. Samples with a minimum of 50 ng of DNA were put through library preparation for next generation sequencing using a Kapa Hyper Prep Kit (Kapa Biosystems).

The Kapa Kit protocol consists of an end repair/A-tailing step, followed by an adapter ligation step, a post ligation bead clean using Agencourt AMPure XP beads, a size selection step using Agencourt SPRI (Agencourt Bioscience Corporation, Beckman Coulter) beads, a library amplification step, and a final post-amplification bead clean. Libraries were size selected for 300-500 bp fragments (Toonen et al. 2013). The concentration of adapters as well as number of amplification cycles varied based on input DNA quantity (Kapa Hyper Prep Kit). Illumina Tru-seq duel index high throughput adapters were used for all libraries. Each library was then quantified with the Qubit 3.0 fluorometer. Sequenced libraries contained a minimum concentration of 1 nM DNA/µl.

DNA Sequencing

Libraries were sent to the Genomic Services Lab at Hudson Alpha (Huntsville, AL) and the QB3 Vincent J. Coates Genomics Sequencing Laboratory at the University of California at Berkeley. All libraries passed quality controls (Bioanalyzer) before being sequenced. Three libraries were used to de novo assemble a mock reference and were sequenced on an Illumina Mi-Seq analyzer (Illumina, Inc.) to obtain longer (300 bp paired end) reads to increase the number of overlapping paired reads and the quality of the de novo assembly. All remaining libraries were sequenced (100 bp paired ends) on an Illumina Hi-Seq2500 or Illumina Hi-Seq4000 analyzer (Illumina, Inc.). After extraction and library preparation, 34 libraries were sequenced for analysis. In total, 18 libraries were successfully sequenced from bleached corals (4 Kāne‘ohe, 9 Lanikai, 5 Waimānalo) and 16 libraries were successfully sequenced from unbleached corals (2 Kane‘ohe, 8 Lanikai, 6 Waimānalo).

Assembly and SNP Discovery

Sequencing returned an average of ~4.3 million de-multiplexed reads per individual. Adapters were trimmed using CUTADAPT (Martin 2011) and paired-end reads were merged and validated with the CombinePairedEnd python script (https://github.com/enormandeau/Scripts/blob/master/fastqCombinePairedEnd.py) for each individual library. VSEARCH v1.0.16 (Rognes et al. 2016) was then used to dereplicate, sort, and assemble a de novo mock reference from the 12.3 million paired and merged reads returned from the three Mi-seq libraries. VSEARCH was used to cluster centroids and further dereplicate, sort, and cluster the clusters from before. The resulting mock reference contained approximately 2.28 million contigs for which a reference dictionary was created with PICARD TOOLS v1.140 (http://broadinstitute.github.io/picard/). All of the other individual libraries which passed quality

filters (two libraries failed and were removed from further analyses) were assembled to the de novo reference file with BWA v0.1.11 using the MEM algorithm (Li 2013). SAM files were converted to BAM files using SAMTOOLS v1.2 (Li et al. 2009). The subsequent BAM files were sorted and filtered using SAMTOOLS to remove alignments that were not properly mapped, paired, or had mapping quality scores less than 30. Filtered BAM files were merged with BAMTOOLS v2.3.0 (http://github.com/pezmaster31/bamtools) and GENOME ANALYSIS TOOLKIT (GATK) v3.4-46 (McKenna et al. 2010) was used to realign mappings around INDELS. FREEBAYES v0.9.21 (Garrison and Marth 2012) was used to call SNPs (freebayes -0 –E 3 –C 4 –G 10 –z .1 –X –u –n 4 –min-coverage 10 –min-repeat-entropy 1 –V) and VCFTOOLS v0.1.11 (Danecek et al. 2011) was used to filter out SNPs with a depth greater than or equal to 20. These SNPs were filtered further with VCFTOOLS for sites that were genotyped in 90% of individuals (--max-missing 0.9), had a minimum quality score of 20 (--minQ 20), a minimum and maximum non-reference allele frequency of 0.05 and 0.95 respectively (--non-ref-af 0.05 –max-non-ref-af 0.95), and a minimum minor allele frequency of 0.05 (--maf 0.05), and removed sites that included indels (--remove-indels). These filters returned a dataset of 46,759 SNPs. Additionally, SNPs used for pairwise Fst and heterozygosity tests and STRUCTURE analysis were further filtered to only include sites that were within a specified distance from each other (--thin 300), and this additionally filter returned 10,528 SNPs. SNPs were called on all data based on phenotypic groupings (bleached or non-bleached) as well as geographic populations (Kāneʻohe Bay, Lanikai, Waimānalo).

Population Structure

Vcf files were converted to GENEPOP and STRUCTURE format using PGDSpider v2.0.9.0 (Lischer and Excoffier 2012). Population wide pairwise F_{st} values were computed for

geographic populations and population differentiation (genic) was tested through Fischer's exact tests for all population pairs using GENODIVE v2.0b23 (Meirmans and Van Tienderen 2004). Population structure was assessed using the program STRUCTURE v2.3.4 (Pritchard et al. 2000) on SNP datasets using a burn in period of 10,000 and 100,000 reps under the ADMIXTURE population model with the LOCPRIOR option (Hubisz et al. 2009) The LOCPRIOR model (Hubisz et al. 2009) accommodates low sample size and weak structure by assessing whether specified population groupings match the trends in ancestry and discarding the population groupings if they don't match. The benefits of the LOCPRIOR model are that it doesn't find structure when none is present and it can ignore the supplied LOCPRIOR data if it doesn't correlate with ancestry results. The Evanno Method (Evanno et al. 2005) was used to determine the appropriate value of k, and analysis was based on STRUCTURE HARVESTER results (Earl et al. 2012).

Population structure was also assessed through Discriminant Analysis of Principal Components (DAPC-Jombart et al. 2010). All discriminant functions were retained for analysis because of the low number of groupings, so k = n - 1. To determine the number of principal components retained a cross validation analysis was run using the default parameters, where 90% of the data is used as a test dataset to predict the placement of individuals into groups for the remaining 10% of the data. The number of principal components retained were selected based on the lowest root mean square error (RMSE). DAPC produces an assignment score for each individual for each population relating to the strength of the association of that individual with that population. Average correct assignment was computed for each population, where a DAPC score > 50% for an individual's true population was scored as correct assignment. The strength of the analysis was examined by randomly assigning populations to individuals, running a

DAPC, and computing a correct assignment from random population designations. This process was repeated for 1,000 permutations and mean correct assignment scores were calculated for each population. The analysis was carried out in R v3.3.2 (R Core Team 2016) using the following packages: adegenet (Jombart 2008; Jombart and Ahmed 2011), adegraphics (Dray et al. 2017), pegas (Paradis 2010), ape (Paradis et al. 2004), and vcfR (Knaus and Grunwald 2016, 2017).

Phenotypic Discrimination

Vcf files were converted to GENEPOP, GENETIX, and BayeScan format using PGDSpider v2.0.9.0 (Lischer and Excoffier 2012). A group wide pairwise F_{st} value was calculated for the phenotypic groupings and differentiation between phenotypes was tested through calculation of Pairwsie F_{st} values for each locus and Fischer's exact tests as described above using GENODIVE (Meirmans and Van Tienderen 2004). Tests for differences in heterozygosity between phenotype groupings were run using the R package adegenet (Jombart 2008; Jombart and Ahmed 2011). Outlier analysis was run on the filtered SNP dataset with groupings determined by phenotype using the Bayesian simulation method developed by Beaumont and Balding (2004) applied in BayeScan2.1 (Foll and Gaggiotti 2008). This program uses a locus effect and population effect in a logistic regression model, with explanations for patterns from locus effects considered for divergent selection. An outlier analysis was run for 100,000 iterations with a burn in of 50,000 iterations, a prior odds value of 10, and 5,000 pilot runs (Lemay et al. 2015). Outlier loci were identified using a false discovery rate (FDR) of 0.20. Downstream analysis was completed in R v3.3.2 (R Core Team 2016) with BayeScan2.1 provided R code. Additionally, phenotypic discrimination was assessed through DAPC and k = n-1. Cross validation was used to determine the number of principal components to retain, as

described above. Percent correct assignment was also computed for each phenotypic group, along with the percent correct assignment for 1,000 DAPC permutations with individuals randomly assigned to phenotypic groups.

Results

Population structure

Population wide pairwise F_{st} values indicate Lanikai showed the most differentiation from other locations, in particular from Kāneʻohe Bay (F_{st} = 0.024, Fisher's exact p-value = 0.001), and overall there was significant population differentiation detected between all locations (Table 3.1). STRUCTRUE HARVESTER results indicated the optimal value of k was two based on ΔK values. STRUCTURE analysis for all individuals (Kāneʻohe Bay, Lanikai, Waimānalo) revealed population structure between the three windward Oʻahu locations (Figure 3.2). Individuals from Kāneʻohe Bay and Lanikai formed distinct clusters, and individuals from Waimānalo clustered mostly with Kāneʻohe Bay. DAPC returned near perfect discrimination between locations (Figure 3.3a), as the mean percent of correct assignment for all populations was 96.9% (Figure 3.4a). The population discrimination with DAPC was robust to randomization of individual genotypes, as the mean classification rate for randomized data (±se) was 48.10% (±0.51).

Phenotypic Discrimination

In contrast to patterns of population subdivision above, estimates of F_{st} showed no significant genetic differentiation among bleaching phenotypes (F_{st} = 0.002, Fischer's exact p-value = 0.13), and no significant differences in genetic variability were found from tests of heterozygosity between all individuals based on phenotype (500 simulations, H_E estimate =

0.0008, p-value = 0.946). The outlier analysis implemented in BAYESCAN2.1 (Foll and Gaggiotti 2008) did not detect any outlier loci under directional selection between bleached and non-bleached phenotypes across all individuals (Kāneʻohe Bay, Lanikai, and Waimānalo combined) (Figure 3.5a) using a FDR of 0.20. The average F_{st} value across all loci was 0.0002 (Figure 3.5b) and the average q value was 0.908, with both metrics indicating no signal of directional selection. The calculated q value acts as a FDR analogue to the p-value and defines the minimum FDR at which a locus will become significant (Foll and Gaggiotti 2008).

DAPC returned moderate discrimination between bleached and non-bleached individuals (Figure 3b) with the percentage of individuals correctly assigned their true bleaching classification was 88.23% and 71.4% for bleached and non-bleached individuals, respectively (Figure 4b). However, this result was not robust to randomization, as the mean (±se) correct assignment to randomized population was 74.46% (±0.25).

Discussion

Thermal history plays a role in thermal tolerance and adaptive capacity in corals, and populations of thermally-tolerant corals exist on some reefs as a result of local environmental conditions (Castillo et al. 2012; Kenkel et al. 2013a, 2015a; Schoepf et al. 2015; Thomas et al. 2018). Bleaching often differentially affects neighboring colonies within reefs, which suggests standing genetic variation for bleaching tolerance within populations, particularly when symbiont compositions of differentially bleached neighbors are similar or symbiont shuffling does not occur (Goulet 2006; Kenkel et al. 2013b; Cunning et al. 2016). The 2014 bleaching event in Hawaiʻi presented a unique opportunity to investigate individuals expressing differing bleaching responses in the same habitat with no recent history of extreme thermal stress. In my study,

differential bleaching patterns suggested acclimatization or adaptation was present in the coral; therefore, we controlled for symbiont type, and only found one individual from Kāneʻohe Bay harboring thermally tolerant clade D *Symbiodinium*. *Montipora capitata* is known to mostly associate with clade C *Symbiodinium* in Hawaiʻi (Stat et al. 2013), but has been linked to higher proportions of clade D in Kāneʻohe Bay relative to other locations in the archipelago. In individuals harboring thermally sensitive clade C *Symbiodinium*, we found no signals of selection within the genomes of *M. capitata*, although we did find underlying population structure between the windward Oʻahu sampling locations.

The most likely explanations for the lack of genetic associations with bleaching phenotypes are: first, the populations I examined may lack sufficient standing genetic variation for selection to act upon, or, second, if there is existing variation we did not detect it. In the first case, while high gene flow across broad geographic ranges has been found for a number of coral species (Rodriguez-Lanetty and Hoegh-Guldberg 2002; van Oppen et al. 2008; Baums et al. 2012), there is increasing evidence that pelagic larval duration and dispersal distance are only one part of understanding genetic connectivity in marine populations (Selkoe and Toonen 2011). *Montipora capitata* in Hawaiʻi has low connectivity across the spatial scale of the Hawaiian archipelago, with evidence for self-seeding within islands (Concepcion et al. 2014). Additionally, Hawaiʻi is isolated geographically and located at the upper latitudinal limit of the tropics and as a result bleaching thresholds for Hawaiian corals are below those for corals located at lower latitudes (Jokiel and Coles 1990). Given that Hawaiian corals had not previously been exposed to extreme thermal stress prior to the 2014 bleaching event, it is also conceivable that geographic isolation and low gene flow between islands resulted in limited genetic variation on which selection for thermally tolerant alleles might act.

In the second case where existing genetic variation was present between the phenotypes, but undetectable, it is likely a result of the small additive effects of many genes (Kenkel et al. 2013a; Rose et al. 2016; Seneca and Palumbi 2015; Louis et al. 2017; Traylor-Knowles et al. 2017a, 2017b). As such, any signals of selection are expected to be weak when spread across many loci (Savolainen et al. 2013; Berg and Coop 2014; Tiffin and Ross-Ibarra 2014). Additionally, sample size was low due to difficulty with DNA extractions and subsequent library preparation. As a result, our statistical power was low for most analyses, particularly for DAPC, as the number of principal components retained in the analysis is limited by sample size and cannot exceed n/3. The DAPC did not discriminate between individuals of different phenotypes, as the measure of success when assigning random phenotypic groupings were similar, but perhaps if sample sizes were higher it would have increased the robustness of this analysis. And finally, RADseq methods produce a reduced representation of the full genome and while they are a very useful tool for working with non-model organisms (Toonen et al. 2013), it is possible that signals of selection could be missed in areas of the genome not sequenced (Stanton-Geddes et al. 2013; Tiffin and Ross-Ibarra 2014).

In addition, if there are in fact no consistent genetic differences between colonies that expressed differential bleaching phenotypes, then phenotypic plasticity likely contributed to the responses of some individuals (Bruno and Edmunds 1997; Todd 2008). While we attempted to control for micro-habitat differences by collecting individuals of similar size within 4.5 m of each other on the reef that exhibited stark differences in bleaching, small differences in light exposure, water flow, and sedimentation stress may have impacted an individual's response. Differences in light exposure on different sides of *Goniastrea aspera* colonies influenced bleaching responses, with the more exposed sides of colonies better acclimatized to

photosynthetic stress as indicated by their better performance under subsequent thermal stress conditions (Brown et al. 2002a). Evidence obtained on survivorship of *Acropora digitifera* on Ryukyu Island, Japan during a 1998 bleaching event showed differences in colony response were mediated by flow rates around the colonies. Colonies in areas with higher flow rates bleached less despite the fact that all colonies were located within a few kilometers of each other and experienced similar thermal stress and light regimes. Additionally, for individuals of *Montipora peltiformis* on the Great Barrier Reef experienced reduced photochemical efficiency and zooxanthellae density in response to sedimentation cover (Philipp and Fabricius 2003). With a loss of zooxanthellae during bleaching, sedimentation stress could further exacerbate the loss of photosynthetically acquired nutrients. As such, small-scale environmental variation may have influenced bleaching responses even under identical levels of thermal stress.

Additionally, while we controlled for the dominant symbiont type, the role of rare *Symbiodinium* in influencing bleaching response has yet to be fully understood, and may have contributed to differential thermal stress responses (Boulotte et al. 2016). Along with the possible effect of a rare algal symbiont is the unknown role of the other organisms associated with the coral animal. In particular, the microbiome community is an important component of the coral holobiont (Ainsworth et al. 2015; Sogin et al. 2017) and may play an influential role in bleaching responses (Gilbert et al. 2012; Ziegler et al. 2017; Pootakham et al. 2018), but there is little known on its effect on the coral bleaching process (Bourne et al. 2016; Webster and Reusch 2017).

Finally, given the population structure identified in the windward O‘ahu locations and the fact that we did not have the power to discriminant phenotypes within each population, the population structure may have overshadowed any weak signals of selection for thermal stress

tolerance in the genome. Previous work examining the population structure of *M. capitata* throughout the Hawaiian archipelago identified four major genetic groupings, with the Main Hawaiian Islands clustered into one group (Concepcion et al. 2014). Our data indicate fine scale population structure for within-island populations of *M. capitata*, which coincides with predictions from Concepcion et al. (2014) based on low migration rates and self-seeding within islands. In order to determine more fine scale spatial patterns of genetic structure within O‘ahu populations of *M. capitata*, more in-depth sampling across more sites on O‘ahu is needed.

In summary, genome-wide comparisons using RADseq data provided little evidence of an underlying genomic basis for inter-individual variation in coral bleaching phenotypes in *M. capitata*. Outlier analysis, population genetic tests, and DAPC analysis could not significantly differentiate between individuals from bleached or non-bleached phenotypes and no signals of directional selection were detected, which is not surprising given the geographic isolation of the Hawaiian archipelago and its long history without bleaching events. However, this has important implications for the adaptive capacity of *M. capitata* in the future, particularly in light of changing environmental conditions. We did find population structure within O‘ahu populations of *M. capitata*, adding more detail to the existing knowledge of its population structure throughout the Hawaiian archipelago. More studies are needed to parse out what physiological or genomic variation might be driving differing bleaching responses in individuals under identical stress conditions. Given the predictions for increased thermal stress in the future (Hughes et al. 2017), it is important to fully understand the coral thermal stress response and whether populations will be able to adapt or acclimatize to increasing environmental change.

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Tables and Figures

Table 3.1: Pairwise F_{st} values (and corresponding Fischer's exact p-values) for tests of genetic differentiation for each location combination between Kāneʻohe Bay, Lanikai, and Waimānalo.

	Kāneʻohe Bay	Lanikai
Lanikai	0.024 (0.001)	
Waimānalo	0.018 (0.003)	0.011 (0.001)

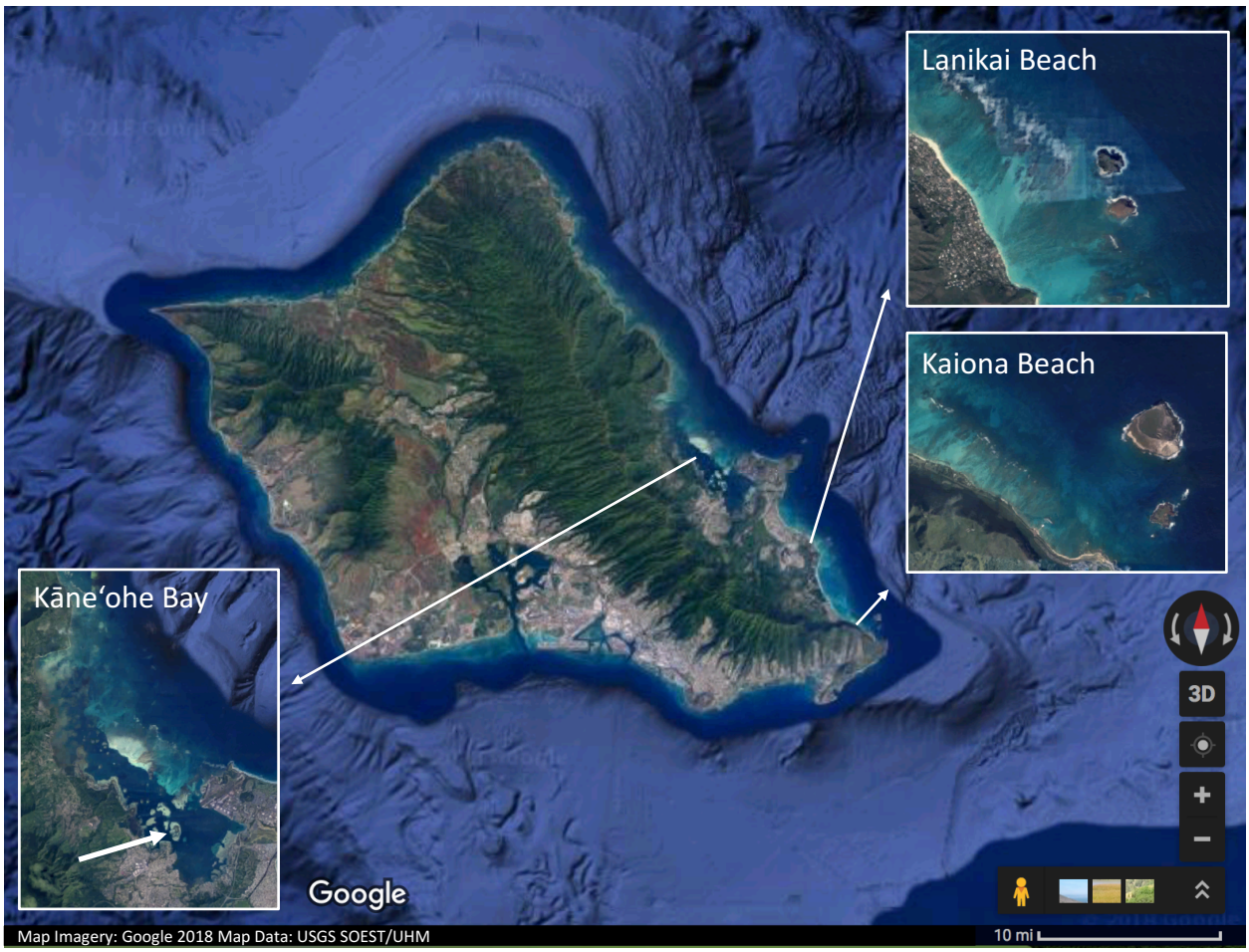

Figure 3.1: Map of sampling locations on Windward Oʻahu: Kaneʻohe Bay, Lanikai Beach (Lanikai), and Kaiona Beach (Waimānalo). The thick arrow in the Kāneʻohe Bay inset points to the Hawaiʻi Institute of Marine Biology.

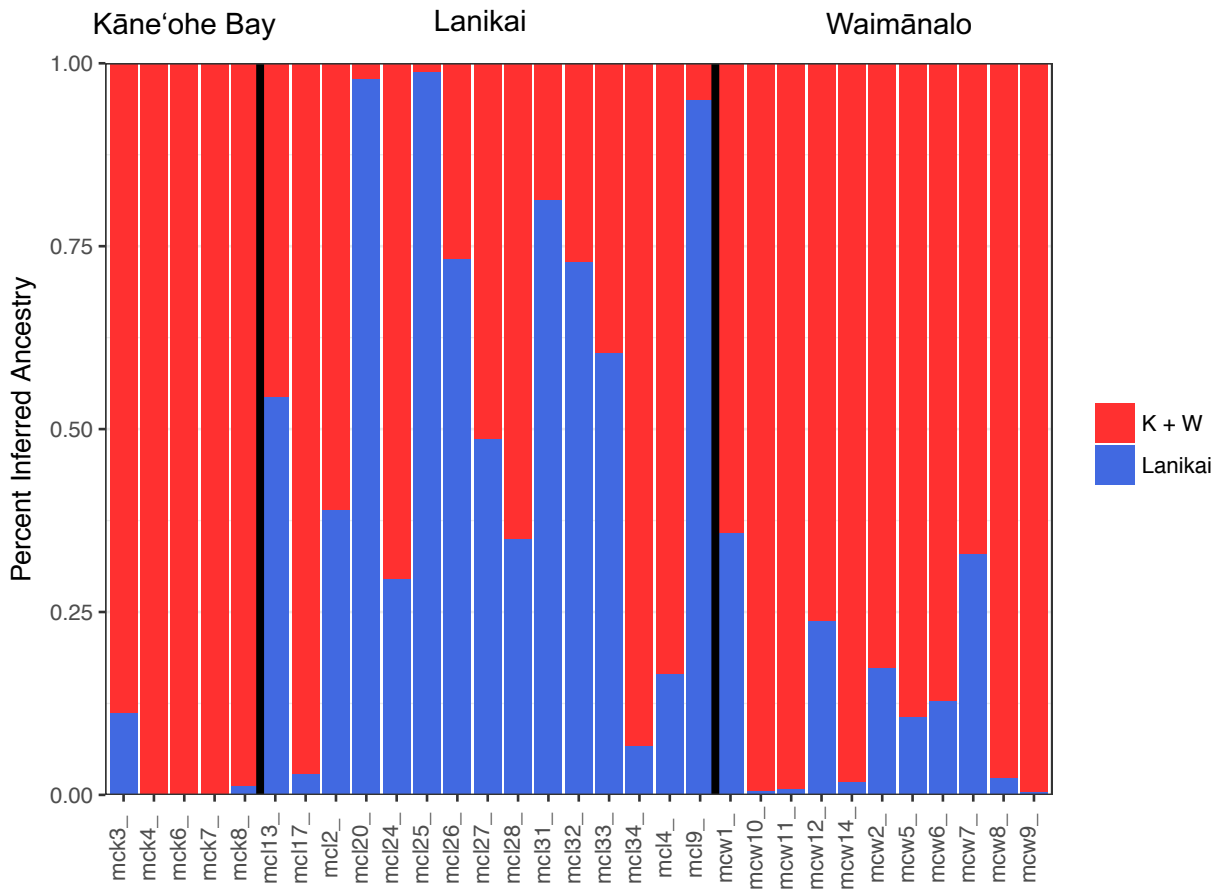

Figure 3.2: STRUCTURE results showing inferred ancestry clusters for Windward Oʻahu populations (k = 2).

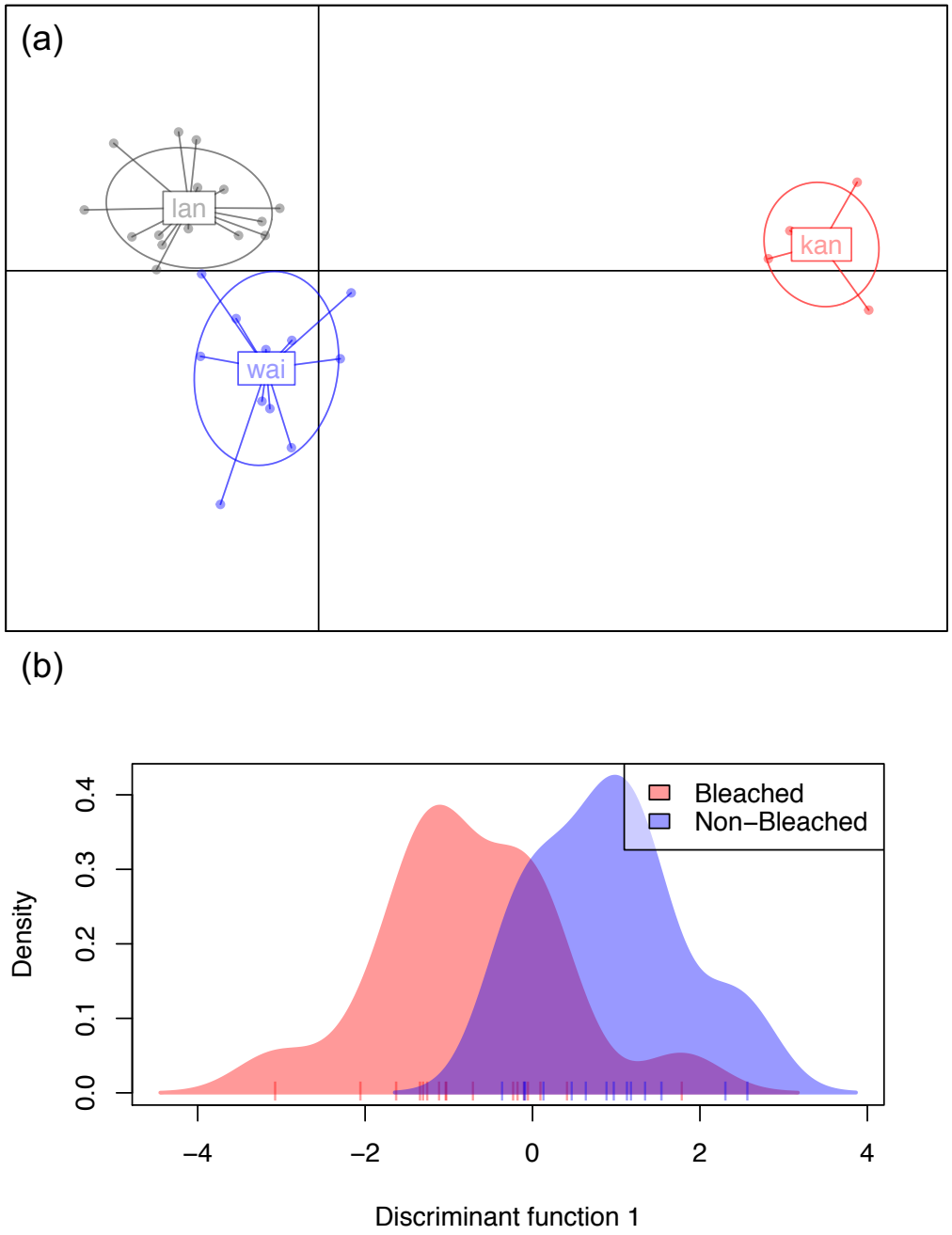

Figure 3.3: Scatter plots from the DAPC for individuals from all locations (Kāneʻohe Bay, Lanikai, Waimānalo) with discrimination based on (a) geographic populations and (b) phenotypes. (Number of discriminant axes = k-1 and therefore (a) DA = 2 and (b) DA = 1)

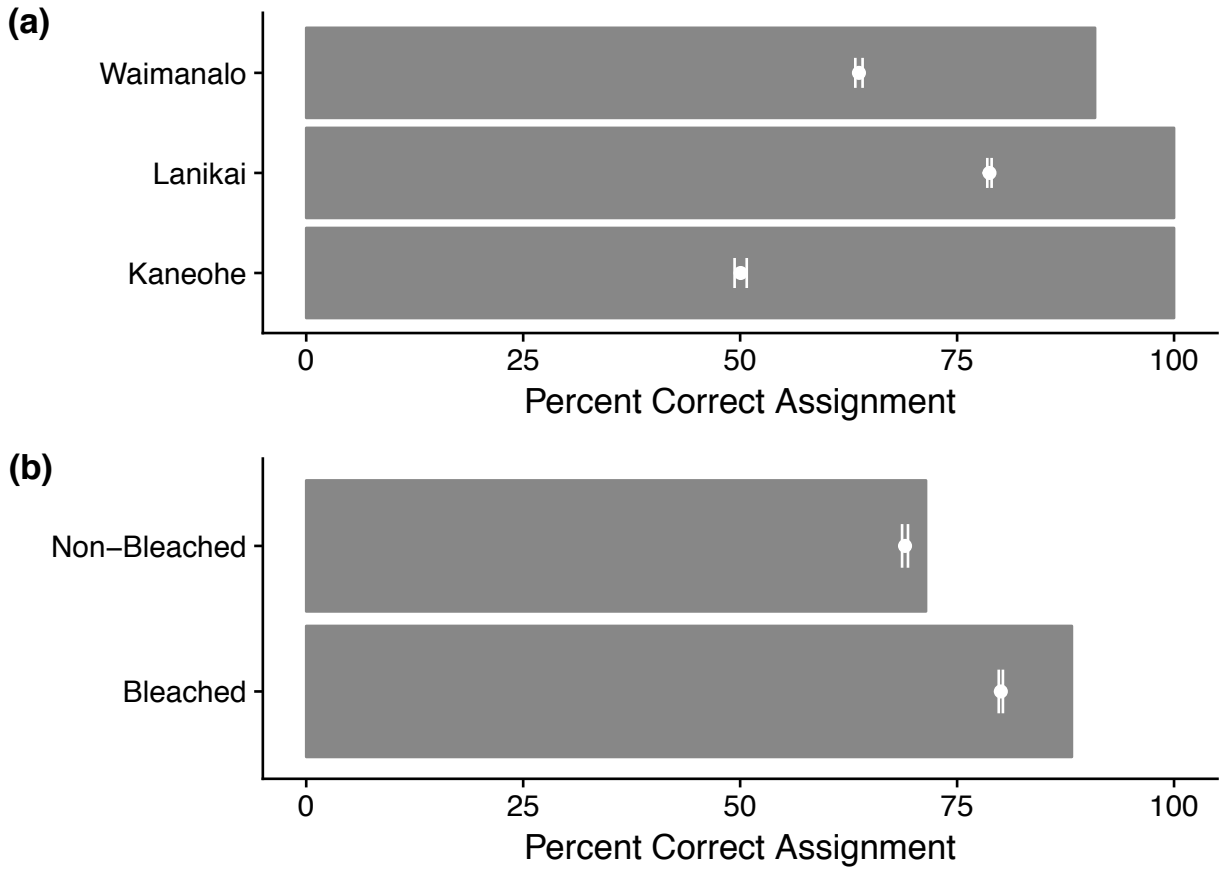

Figure 3.4: Percent correct assignment from DAPC (bars) with the mean percent correct assignment (±se) from 1000 DAPC permutations with random population assignment for discrimination based on (a) geographic populations and (b) phenotypes.

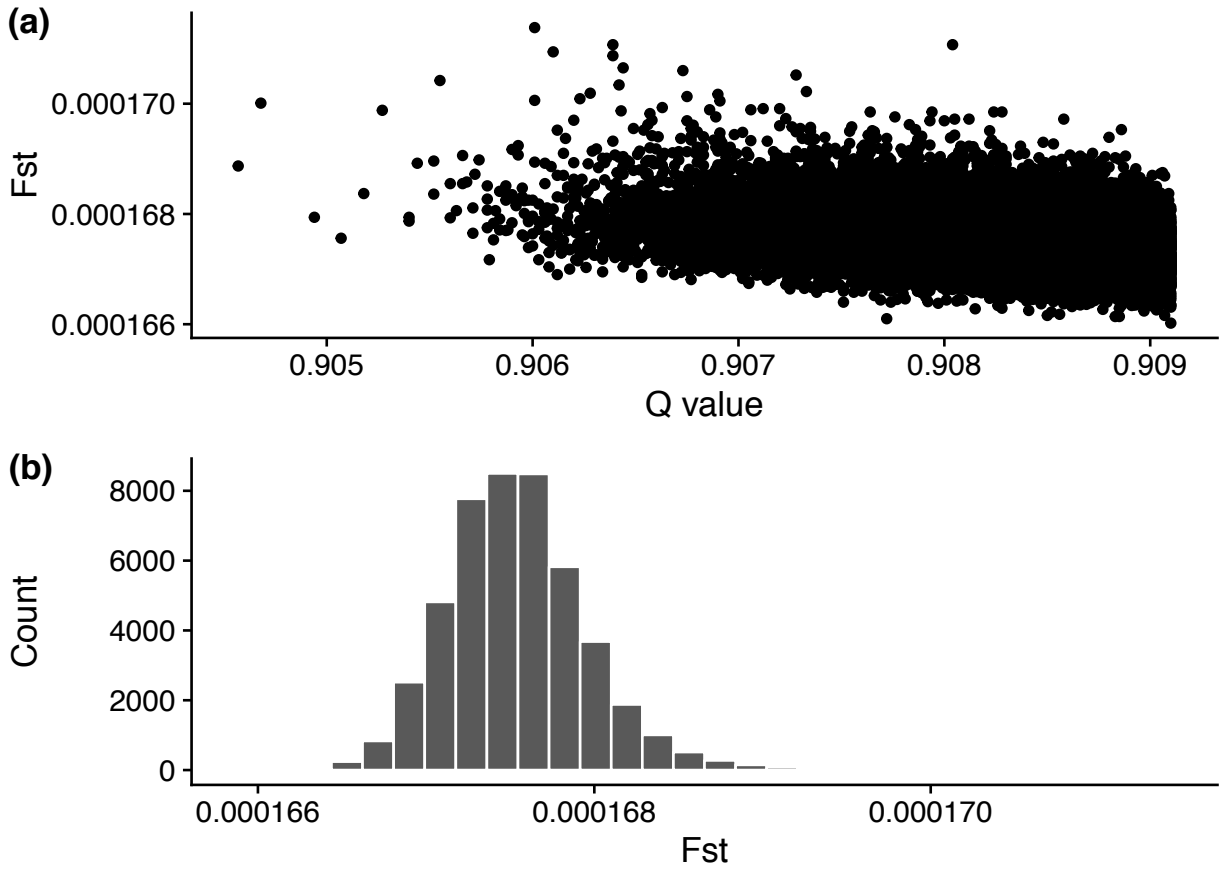

Figure 3.5: (a) Bayescan2.1 calculated qvalues (proxy p-values) by F_{st} for each loci and (b) a histogram showing the distribution of F_{st} values throughout the SNP dataset.

CHAPTER FOUR

LIPID STORAGE AND REPRODUCTION IN CORAL GROWTH ANOMALIES IN *PORITES EVERMANNI*

Abstract

Growth anomalies (GAs) are protuberant masses found on many coral species and their presence may be linked to increased ocean temperatures and bleaching stress. In 2014 and 2015 Hawai‘i experienced back-to-back beaching events, after which an increased number of growth anomalies (GAs) were noted on massive *Porites* colonies. GAs and normal tissues were sampled from each of 15 *P. evermanni* colonies on six dates from June 2016-August 2017, encompassing two annual reproductive cycles. Compared to normal tissue, GAs had significantly larger corallites in all dimensions and reduced amounts of lipid and lower functional lipid ratios across all time points. The latter may be attributable to the faster growth rate of GAs, which could lead to mobilization rather than storage of energy rich lipids. Lipid content was generally higher in winter (December) and spring (April) than in summer months (June/July), but was not correlated with reproductive condition. Surprisingly, GA tissues from 2017 contained elevated numbers of oocytes. Our data confirm that GAs in *P. evermanni* have abnormal corallite morphology, as in other species studied, and compared to normal tissues, commit proportionally more energy to growth and less to energy storage. However, unlike other coral species studied to date, GAs in *P. evermanni* may continue to contribute substantially to overall colony gamete production, although with delayed timing or questionable viability.

Introduction

Thermal stress as a result of global climate change is predicted to increase disease susceptibility in organisms (Harvell et al. 2002; Bruno et al. 2007), and links between anomalously warm temperatures and disease outbreaks have been established for a number of marine and terrestrial species (reviewed in Harvell et al. 2002). In addition, in corals, elevated temperatures have been

linked to stress responses such as coral bleaching (Coles et al. 1976; Jokiel and Coles 1990) as well as disease (Bruno et al. 2007; Muller et al. 2008; Heron et al. 2010; Maynard et al. 2011), both of which threaten the fitness and ecological health of the coral and ultimately the surrounding reef (Pandolfi et al. 2003; Bellwood et al. 2004; Hughes et al. 2017).

Growth anomalies (GAs) (Figure 4.1) are a common coral disease characterized by protuberant masses (Hunter 1999; Aeby et al. 2011), often with decreased skeletal density and zooxanthellae concentrations (Peters et al. 1986; Yamashiro et al. 2001; Gateno et al. 2003; Burns and Takabayashi 2011; Burns et al. 2011). Growth anomalies have been classified as neoplasia (White 1965; Squires 1965; Bak 1983; Peters et al. 1986), hyperplasia (Willis et al. 2004), calicoblastic epitheliomas (Coles and Seapy 1998), and tumors (Cheney 1975; Loya et al. 1984; Yamashiro et al. 2001). They have been documented in 41 species across 18 genera throughout the Caribbean and Indo-Pacific (Sutherland et al. 2004).

As with many coral diseases, the etiology of GAs is not fully understood, but they have been linked to bacterial infection (Domart-Coulton et al. 2006), parasitic endolithic algae (Le Campion-Alsumard et al. 1995; Coles and Seapy 1998; Domart-Coulton et al. 2004; Breitbart et al. 2005; Work et al. 2008), high intensity ultraviolet light (Loya et al. 1984; Peters et al. 1986; Coles and Seapy 1998), and ageing (Irikawa et al. 2011). Prevalence of growth anomalies has also been associated with human population density (Aeby et al. 2011), density of coral cover (Bruno et al. 2007), and bleaching events (McClanahan et al. 2009; Stimson 2011; Cantin and Lough 2014; Mallela et al. 2015). High seawater temperatures and environmental changes commonly associated with bleaching events (decreased water flow, increased light penetration, decreased nutrients) and lower zooxanthellae density may result in a reduction in coral calcification, leading to the invasion of endolithic fungi (McClanahan et al. 2009). Endolithic

fungi may cause skeletal structural changes in corals like those seen in coral growth anomalies (Le Campion-Alsumard et al. 1995; Domart-Coulton et al. 2006).

While GAs have been predominantly defined by abnormal morphology, there is increasing evidence for physiological differences between GAs and normal tissue. GAs have faster growth rates (Bak 1983; Cheney 1985; Peters et al. 1986), lower reproductive output (Yamashiro et al. 2000; Domart-Coulton et al. 2006; Work et al. 2008; Burns and Takabayashi 2011), and decreased photochemical efficiency compared to zooxanthellae in normal tissues (Burns and Takabayashi 2013). Tissue lipid stores, as well as the "functional lipid ratio" (the ratio of energy rich storage lipids to structural lipid) have been used to assess the nutritive condition of corals. In general, hermatypic corals rely on photosynthetically fixed carbon from zooxanthellae to meet their metabolic needs (Muscatine et al. 1981; Edmunds and Davies 1986; Grottoli et al. 2006), typically storing excess fixed carbon as lipids. These lipids generally constitute 9-40% of a coral's biomass (Stimson 1987; Harland et al. 1992, 1993; Yamashiro et al. 1999). There are two main groups of lipids in corals: energy rich storage lipids (nonpolar), most notably wax esters (WE) and triacylglycerols (TG), which make up approximately 40-73% of total lipids; and structural (polar) lipids, most notably sterols (ST) and phospholipids (PL), which constitute approximately 9-60% of total lipids (Harland et al. 1993; Yamashiro et al. 1999, 2005; Oku et al. 2002; Rodrigues and Grottoli 2008; Imbs 2013).

Quantity and composition of lipids can vary in response to a number of seasonal factors, including increased lipid content with warmer temperatures and higher light availability (Crossland et al. 1980; Stimson 1987; Oku et al. 2003), decreased lipid content at higher turbidities (Anthony and Fabricius 2000), and decreased lipid content with increasing depth (Harland et al. 1992), as well as changes in lipid composition based on metabolic rates of

photosynthesis and respiration (Imbs 2013), and gonad production (Arai et al. 1993). Processes that deplete storage lipids, such as decreased photosynthesis as a result of bleaching, can also lead to lower production of oocytes as well as decreased mucus production (Crossland et al. 1980), which affects coral defenses (Ritchie 2006; Shnit-Orland and Kushmaro 2009) and energy flow to the reef ecosystem (Coles and Strathmann 1973; Benson and Muscatine 1974; Wild et al. 2004). Only one study to date has examined lipid levels in GAs, and lower lipid stores and less energy rich lipids relative to structural lipids (although an exact ratio was not calculated) were found in *Montipora informis* GAs in Japan (Yamashiro et al. 2001). These results were interpreted to mean the lower energy rich lipids in GAs were a result of increased energy demand to maintain faster growth.

In Hawai‘i, growth anomalies have been found in six species of corals across three families; the most commonly affected are species in the genus *Porites*. *Porites* GAs were first described in Hawai‘i in Hanauma Bay (Hunter 1999) and have since been studied in more detail in branching *P. compressa* in Kāne‘ohe Bay, O‘ahu and on Hawai‘i Island (Breitbart et al. 2005; Domart-Coulon et al. 2006; Takabayashi et al. 2008; Stimson 2011), in massive *P. lobata* and *P. lutea* on Hawai‘i island (Takabayashi et al. 2008; Couch et al. 2014), and in *Porites* spp. as part of a study across the Indo-Pacific (Aeby et al. 2011). Additionally, *Porites* GAs have also been studied in Kenya (McClanahan et al. 2009), the Caribbean (Sutherland et al. 2004), and the Indo-Pacific (Kaczmarsky and Richardson 2007; Williams et al. 2011). However, little is known about how growth anomalies differ physiologically or reproductively from normal tissue, and growth anomalies in *P. evermanni*, a common reef-building species in Hawai‘i, have never been characterized. Therefore, I compared skeletal morphology of growth anomalies to normal tissue in *P. evermanni* and, for comparison, in the sympatric congener *P. lobata*. I then measured

energy reserves and reproductive effort in both growth anomalies and normal tissue in 15 massive *P. evermanni* colonies*;* I further tracked these metrics over two years to gain a fuller picture of the physiological associations between GAs and their host colonies.

Methods

Coral Collection

Samples were collected in 2015, 2016, and 2017 from A‘alapapa reef off of Lanikai Beach on the windward side of the island of O‘ahu, Hawai‘i. I sampled 19 haphazardly-chosen massive colonies of *Porites* (13 *P. evermanni* and 6 *P. lobata*) located within a lagoon behind a 2.4 kilometer stretch of offshore reef (Figure 4.2). Small (25 cm^3) fragments of both normal tissue and anomalous growth were removed from each colony using a hammer and chisel. Samples for skeletal morphology were only collected in the summer of 2015. These samples were soaked in 50% sodium hypochlorite for 1-2 days to remove tissue, after which the skeletons were rinsed twice with fresh water and allowed to air dry for 24-48 h. Separate samples for histological analysis of reproductive state were collected from both GA and normal tissue in 15 massive *P. evermanni* colonies in 2016 and 2017. Full moon is a spawning cue for *P. evermanni* in Hawai‘i during summer months (Richmond and Hunter 1990; Neves 2000). In order to examine reproductive trends, samples were taken in June 2016 and July 2017 prior to the full moon and in August 2016 and August 2017, after the full moon. The two sampling years represent coral condition one and two years after back-to-back bleaching events in 2014 and 2015. Samples were preserved in 1:4 Z-fix:filtered sea water and stored for further processing. Samples for lipid analysis were collected from the same 15 *P. evermanni* colonies on each date. I also collected samples for lipids from the same colonies in December 2016 and April 2017 to

obtain lipid profiles during the non-reproductive season. Samples for lipid analysis were frozen in liquid nitrogen immediately after collection and stored at -80 ^{0}C under N_2 until further processing. All samples were collected using hammer and chisel then further split into two subsamples, one for histological analysis and one for lipid analysis. All samples were collected under State of Hawai‘i Division of Aquatic Resource permits: SAP 2015-47, SAP 2016-74, and SAP 2017-49.

Skeletal Morphology

Photographs of corallites from each sample were taken using a Nikon Coolpix 4500 attached to a dissecting microscope. Each picture was taken at 18x magnification with a 0.08 mm diameter human hair used as a reference in each photo. At least three photos were taken of each sample to acquire 10 clearly imaged corallites for skeletal trait analysis (Forsman et al. 2015). For each of 10 corallites per sample, 24 X-Y coordinates were mapped onto landmark skeletal structures in a clockwise and inward fashion starting from the outside edge of the dorsal directive (see Appendix B: Figure S4.1 for corallite coordinate map). The distance between each coordinate was calculated and the combinations of these X-Y coordinates were used to measure the lengths and distances of 40 corallite traits relating to septal lengths and their relative position. These measurements included pali distances, septal distances, septal lengths, fossa width, fossa length, overall width, and overall length (see Appendix A: Table S4.1 for list of traits and corresponding X-Y coordinates). The terms “width” and “length” were used to distinguish the horizontal diameter and vertical diameter of the corallite. For each sample, measurements were averaged across corallites for each of the 40 morphometric calculations. All image analysis was performed in Image J (Schneider et al. 2012).

Dry weight and Ash Free Dry Weight

For each sample, a small (0.9-2.5 g) fragment (tissue + skeleton + zooxanthellae + sample water) of known weight was placed in a mortar and pestle, with DPEC water equal to 1 ml water per g wet weight of coral, and crushed until a homogeneous slurry was obtained (Farre et al. 2010; Conlan et al. 2017). To obtain a dry weight (DW) measure for standardization between samples, we dried two replicates of 100 ul of coral slurry (1 mL DPEC water:1g wet weight crushed coral) in pre-ashed pans at 60 ^{0}C in a drying oven to a constant weight (three hours was sufficient based on pilot studies). Dried samples were then ashed at 450 ^{0}C in a muffle furnace (Thermo Scientific) for two hours (again to a constant weight) and reweighed to obtain ash weight. Ash free dry weight (AFDW) for each pan was calculated as the difference between DW and AW. For each sample, the mean of the two technical replicates was calculated and used as the estimate of DW, AW, and AFDW for that sample.

Lipid Analysis

Because lipid concentration and composition can vary between surface and internal tissues, lipids were extracted and analyzed from whole coral samples ground with a mortar and pestle as above. For dry weight measurements, lipids were extracted from duplicate samples of 100 µl of crushed coral. Lipids were extracted in 2:1 cold methanol:chloroform for 30 minutes at -20 C^0. Extractions were washed in a 1:1 chloroform:water solution and then dried down under a stream of medical-grade N_2 gas and re-suspended in 30 µl of chloroform. Two replicate 1 µl subsamples were then spotted onto quartz-impregnated chromatographic rods (Chromorods, Mitsubishi Chemical Medience Corporation) and run through a two-step development process as described in Rodrigues et al. 2008. For the first step, the rods were developed in 99:1:0.05 (v:v:v) hexane:diethyl ether:fomic acid for 30 minutes and then scanned to 75% of their length

on an Iatroscan MK-6s analyzer (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan). In the second step, the rods were developed in 80:20:0.1 (v:v:v) hexane:diethyl ether:formic acid for 30 minutes and then scanned to 100% of their length. For standards I used palmityl palmitate (Sigma-Aldrich) for WE, methyl stearate (Sigma-Aldrich) for FAME, glycerol tripalmitate (Beantown Chemical) for TG, palmitic acid (Sigma-Aldrich) for FFA, cholesterol (MP Biomedicals, LLC) for ST, L-α-phosphatidylcholine (Sigma-Aldrich) for PL, and 1-Octadecanol (Sigma-Aldrich) for an internal standard.

Along with quantification of individual classes, the total percent lipid of each sample was calculated as the total lipid divided by the AFDW for that sample. Additionally, I calculated the ratio of storage, or energy rich lipids (WE, FAME, TG, and FFA) to structural polar (ST and PL) lipids (Oku et al. 2003; Saunders et al. 2005; Cooper et al. 2009, 2011; Henrichs et al. 2013a, 2013b). Because storage lipids are known to vary with seasonal changes in environmental variables, while structural lipids are more stable, this functional lipid ratio has been suggested as a more appropriate measure of coral condition and resilience compared to a measure of total lipids (Cooper et al. 2009, 2011). Lipid analysis was performed on all samples from all 15 colonies for the four dates with corresponding histological analysis taken during the reproductive season (June 2016, August 2016, July 2017, and August 2017). To allow for comparison in the winter, when no colonies were reproductive, I chose a subset of eight colonies for lipid class analysis of the December 2016 and April 2017 samples. In total, 152 samples were analyzed for lipid content.

Histology

To determine reproductive status, I compared histological sections of normal and GA tissue from individual colonies over time. Sectioning for histological assessment of gonads was

performed by J. Berger at Histo Techniques; samples were decalcified, trimmed, embedded in paraffin, sectioned at 10 μm, and stained with hematoxylin and eosin. Sections were examined from all samples to determine the sex of each individual and compare their reproductive states. Slides were examined and photographed using an Olympus BX41 compound microscope equipped with an Olympus Q-color3 camera and Q-capture (Q-Imaging) software. Image J (Schneider et al. 2012) was used to measure the area of all gonads (μm^2) and the area of all tissue examined (mm^2). For each slide the area of gonads in μm^2 over the area of coral in mm^2 was calculated, hereafter referred to as the reproductive index.

Statistics

To determine whether normal and GA skeletal structures were morphologically distinct, I used a forward stepwise discriminant analysis to examine the success rate for classification of corallite trait measurements into their respective health category. Coral morphometric data analysis was run in SYSTAT Version 13 (Systat Software, San Jose, CA). All other analysis and graphics were completed using R 3.4.3 (R Core Team 2017). Because the data were non-normally distributed and zero-inflated, reproductive indices were evaluated with Wilcoxon signed-rank tests that compared differences in reproductive effort between dates and health states (GA versus normal).

Generalized linear mixed models were used to examine the effects of health, date, and reproductive index on total lipids and lipid ratios. The total lipid model was run with the negative binomial distribution applied to the response variable within the model using glmer.nb from the R package lme4 (Bates et al. 2015) to correct for non-normality and overdispersion. Overdispersion was checked by examining the Pearson residuals and residual degrees of freedom. I tested for differences between functional lipid ratios using the lognormal distribution

applied to the response variable within the model using glmer from the R package lme4 (Bates et al. 2015) to correct for non-normality. Additionally, the default optimizer "bobyqa" was used to account for failures to converge and start values were specified through the mustart option. Total lipids and functional lipid ratio data were analyzed in separate models, and both models were run with health and date as fixed effects and coral colony as a random effect. These models included data from all six sampling dates.

Because most histological sections contained no visible gonadal tissue, the reproductive index data were zero-inflated. Therefore, I binned those data into three categories: low (0-100 $\mu m^2/mm^2$), medium (101-500 $\mu m^2/mm^2$), or high (>500 $\mu m^2/mm^2$). This three-level metric was included as a fixed effect in additional total lipid and functional lipid ratio models to test for a relationship between reproduction and lipid composition and content. These models used the same structure as mentioned above, but only included data for the four time points for which histological data were available: June 2016, August 2016, July 2017, and August 2017.

For all models, fixed effects were evaluated with parametric bootstrapping with null models using the R package pbkrtest (Halekoh and Hojsgarrd 2014). Multiple comparisons with Tukey adjustments were used to assess variation in lipid content and composition between individual sampling dates and between levels of reproductive index using the R package emmeans (Lenth 2018). Additionally, all models were compared using Akaike information criterion corrected for small sample sizes (AICc) from the R package MuMIn (Barton 2017) to determine which predictors best explained the variation in each dataset.

Results

Skeletal morphology analysis

Morphometric analysis of corallite data revealed GA corallites had larger structural trait distances compared to normal tissue (Figure 4.3). Forward stepwise discriminant analysis showed significant discrimination of normal versus GA corallites for both *P. evermanni* (Wilk's lambda = 0.599, p = 0.001) and *P. lobata* (Wilk's lambda = 0.609, p = 0.001). Corallites were correctly classified as normal 84% of the time for *P. evermanni* and 87% of the time for *P. lobata*, and correctly classified as GA 71% of the time for *P. evermanni* and 77% of the time for *P. lobata*. In *P. evermanni* three septal lengths, two pali distances, and two septal distances drove discrimination. In *P. lobata* four septal lengths, three pali distances, and one septal distance drove discrimination.

Lipid analysis

Overall percent lipid content was low, with an average of 6.42% lipid per g AFDW in normal samples and 4.80% lipid per g AFDW in GA samples. Analysis of lipid datasets revealed significant differences between tissue health and date, but showed no significance to reproductive indices. Total lipids were significantly higher in normal versus GA samples across all dates (Figure 4.4a) for models with and without reproductive indices included ($p < 0.01$). Across both normal and GA samples, total lipids varied significantly by date (Figure 4.5) for models with and without reproductive indices ($p = 0.001$). More specifically, total lipid values were significantly higher in June 2016 than all other summer months (July and August) for models with and without reproductive indices (Table 4.1). Total lipids did not vary significantly with reproductive index ($p = 0.16$) or between any combination of the three index levels. Based

on AICc values the fixed predictors health and date best described the trends in the data, and reproductive indices added no explanatory power to the model.

Lipid classes found in normal and GA tissue of *P. evermanni* were wax esters (WE), triaclyglycerols (TG), free fatty acids (FFA), fatty acid methyl esters (FAME), sterols (ST), and phospholipids (PL). The largest (by mass) lipid classes were WE, TG (energy rich/storage lipids) and PL (structural) (Figure 4.6). PL were overall the most abundant, followed by TG and WE. The remaining lipid classes (FAME, FFA, and ST) made up a smaller percentage of the total lipids. WE and TG together accounted for 36.9% (± 1.9% se) of lipids in normal tissue and 27.4% (± 0.92% se) of lipids in GA tissue; when all energy rich lipids (WE + TG + FAME + FFA) were combined, they made up 59.14% (± 1.43% se) and 50.46% (± 1.11% se) of normal and GA lipids, respectively. ST made up 13.3% (± 0.44% se) and 15.4% (± 0.36% se) in normal and GA tissue and PL made up 27.9% (± 1.21% se) and 34.1% (± 1.21% se) in normal and GA tissue respectively. Together (ST + PL) on average they made up 41.2% (± 1.39% se) in normal tissue and 49.5% (± 1.11% se) in GA tissue.

Across all dates, functional lipid ratios were higher in normal samples than in GA samples (Figure 4.4b); this effect was significant in both functional lipid ratio models (with and without reproductive indices, $p = 0.001$). Lipid ratios varied significantly across dates (Figure 4.7) in both models (with and without reproductive indices, $p = 0.001$), but did not significantly vary between specific dates except for between June 2016 and August 2017 in the model with reproductive indices ($p = 0.01$). Lipid ratios varied significantly by reproductive index level (low, medium, high) ($p = 0.001$), but with no significant differences between any two reproductive index levels. AICc values for both models demonstrated the full model with either both or all three fixed effect predictors best described the trends in the data, meaning tissue

health, time of year, and connection to reproduction all played a role in influencing functional lipid ratios. However, AICc values demonstrated only a slight increase in fit when including reproductive index, and this likely played a minor role in influencing differences in lipid ratios.

Histology

In June of 2016, oocytes were found in both GA (10/15) and normal (15/15) samples from *P. evermanni* colonies, showing that both types of tissue on colonies were reproductive. Reproductive indices were higher in normal versus GA samples in 10 of the 15 colonies (Figure 4.8), but no significant difference in reproductive indices was found in June 2016 between normal versus GA tissues using a Wilcoxon sign-ranked test ($p = 0.45$). In August of 2016, oocytes were found in only one colony in the GA sample. In July of the next year, 2017, oocytes were found in 10 of the 15 colonies (Figure 4.8). Oocytes were again found in both normal and GA samples, with higher reproductive indices in GA versus normal samples in nine of the ten colonies. Significantly more gonad tissue was found in GA samples than in normal samples in July 2017 ($p = 0.008$). In August of 2017, oocytes were found in two normal samples and one GA sample.

Comparing within health state and across dates, there was significantly more gonad tissue in June 2016 versus July 2017 for normal samples ($p < 0.0001$) and significantly less gonad tissue in June 2016 versus July 2017 for GA samples ($p = 0.03$). No significant difference was found in gonad tissue in normal versus GA samples overall ($p = 0.076$) and a test of indices across both tissue health states also showed no significant difference between gonad tissue between June 2016 and July 2017 ($p = 0.89$).

Discussion

Growth anomalies (GAs) on massive *Porites* in Hawai'i showed a consistent signature of differentiation from normal tissue in both skeletal structure and lipid content and composition. I saw consistent patterns of larger calices in GA skeletal corallites compared to normal skeletal corallites in both *P. evermanni* and *P. lobata*. Length and width of corallites were significantly larger in both species, as well, consistent with previous findings for other species of *Porites*: *P. lutea* from Kenya (McClanahan et al. 2009), *P. compressa* in Hawai'i (Domart-Coulton et al. 2006) as well as *Acropora cytherea* in Japan (Irikawa et al. 2011). The traits that varied the least between GA and normal corallites for both species were the pali distances, a finding similar to that reported for interpali lengths in *P. lutea* GAs in Kenya (McClanahan et al. 2009). Earlier reports indicate that the morphology of GAs is not consistent across genera (Work et al. 2008; Burns et al. 2011; Williams et al. 2011). My study of GA morphology in Hawaiian *Porites* expands the range of documented types and our understanding of GAs in *Porites* and its relatives.

Percent lipid composition of our samples (as mg lipid g $afdw^{-1}$) were lower than most found in previously published studies on *Porites* species. Overall, these studies showed the range in scleractinians to be between ~8-58% (Stimson 1987; Harland et al. 1993; Yamashiro et al. 1999; Imbs 2013). Other studies of *Porites* species found that tissues of *P. lutea* were 20.1% lipid (Yamashiro et al. 1999), while lipid percentages from branching *P. compressa*, *P. cylindrica*, and *P. porites* were 11-30% (Harland et al. 1992; Yamashiro et al. 2005; Rodrigues and Grottoli 2007). Ours is the first study to quantify lipids in *P. evermanni*, and our comparatively low percentages (4-7%) may indicate that this species differs from other congeners in normally having low lipid levels.

Alternatively, the bleaching history of *P. evermanni* could have resulted in lipid levels below 'normal' levels. These colonies experienced severe back-to-back bleaching events in 2014 and 2015, two years prior to sampling. Bleaching stress has previously shown to reduce total lipid levels: bleached *P. compressa* and *P. cylindrica* showed lipid levels of 7-12% and 6% respectively (Grottoli et al. 2004; Yamashiro et al. 2005), a greater than 50% reduction over estimates of percent lipid composition in healthy colonies for these species. Additionally, while the colonies in this study were not visibly bleached when samples were taken, they were subjected to the stress of the repeated warming events in the prior two years that may have adversely affected lipid levels in our samples. The most abundant storage lipids in scleractinian corals are WE and TG and the most abundant structural lipids are ST and PL. While overall lipid composition was low, the percent lipid composition of energy rich storage lipids WE and TG (37% normal and 27% GA) were just below the expected ranges (40-73%) and the percent lipid composition of ST and PL (structural lipids) (41% normal and 50% GA) fell well within the range of values published in the literature, 9-60% (Harland et al. 1993; Yamashiro et al. 1999, 2005; Imbs 2013).

Regardless of the possible explanations for our findings, total lipids and functional lipid ratios differed between the two tissue types consistent with the findings of Yamashiro et al. (2001) for *Montipora informis*. Similarly, GAs in *P. evermanni* had lower total lipid content than normal tissue, with the greatest difference seen in WE and TG. While Yamashiro (2001) did not calculate functional lipid ratios directly, I can estimate them from their published values of the percent lipid composition for each lipid class (Yamashiro et al. 2001, Figure 2). Also supporting our findings, the values estimated for functional lipid ratios in Yamashiro et al.'s 2001 study of

M. informis showed higher lipid content in normal versus GA tissue (4.90 for normal tissue and 1.35 for GA tissue) as did ours (1.1-2.5 for normal and 0.9-1.5 for GA).

Increased skeletal extension and tissue growth rates relative to normal tissue is a frequently observed characteristic of GAs (Peters 1986; Gateno et al. 2003; Domart-Coulton et al. 2006; McClanahan et al. 2009). In corals without GAs, rapid growth is associated with lower ratios of energy rich storage to structural lipids, attributed to rapid mobilization of energy rich lipids for faster cell production and growth (Yamashiro et al. 2001; Oku et al. 2002; Denis et al. 2013; Conlan et al. 2018). Lower lipid ratios in GAs may indicate the rapid (and steady) use of energy storing lipids to sustain faster growth than occurs under normal circumstances. Additionally, increased structural lipids (ST and PL) in GAs supports increased growth as ST and PL are the main constituents of cell membranes (Imbs et al. 2010). Alternatively, low levels of lipids could be related to lower densities of zooxanthellae, which has also been frequently observed in GA tissues (Peters et al. 1986; Yamashiro et al. 2001; Irikawa et al. 2011; Williams et al. 2011). This can lead to lower photosynthetic yield (Irikawa et al. 2011) and consequently less energy rich storage lipids.

There was seasonal variation in both lipid metrics as natural seasonal variation exists within coral lipid stores. Some species experience higher tissue biomass, and therefore higher lipid content, in the winter and spring (Fitt et al. 2000) and others experience higher lipid content in the summer and fall (Stimson 1987; Oku et al. 2003). Our results are consistent with those described by Fitt et al. (2000), apart from high total lipids in June 2016, and suggest that increases in temperatures during summer and fall lead to increased respiratory metabolism which uses up lipid stores. However, high functional lipid ratios observed in *P. evermanni* in summer and fall point to more storage lipids during these seasons. Previous work showed positive

correlations between lipid ratios and light and moderate temperature in *Acropora digitifera*, a species which relies mostly on photoautotrophic energy acquisition as does *Porites* (Henrich et al. 2013b). While I do not know why total lipids were low in summer and fall when functional lipid ratios were high, the increased metabolic demand from increased photosynthesis and respiration in the high light summer months used up the newly created energy stores more quickly leading to lower overall lipid stores. Consequently, a decrease in metabolic demand in cooler winter and spring months allowed more energy allocation towards growth, increasing tissue biomass (Fitt et al. 2000) and lipid stores. Given the association of structural lipids with growth (Imbs et al. 2010), an increase in growth and composition of structural lipids would lead to lower lipid ratios, as I observed in winter and spring months in *P. evermanni*

Similar to the seasonal trends in lipid composition, trends in reproductive indices also exhibited unexpected patterns, but provide evidence that there is still more to learn about the reproductive nature of GAs. Given that *P. evermanni* typically spawn at full moon in the summer months in Hawai'i (Richmond and Hunter 1990; Neves 2000), it was not surprising that almost all tissues examined following the full moon in August of both years had no gonads or just a few small oocytes. More surprising, however, was that in June 2016, prior to the likely spawning time, oocytes were present in all 15 colonies but in low numbers and small sizes in most colonies. This suggests a low reproductive output for that year, which likely resulted from the bleaching events in the summers of 2014 and 2015 and resulting depletion of energy reserves. Significant reductions in total lipids as well as energy rich storage lipids (mainly WE and TG) were seen immediately in Hawaiian *Porites compressa* after one month of experimental bleaching (Rodrigues and Grottoli 2007; Rodrigues et al. 2008), and full recovery of pre-bleaching lipid levels did not occur until eight months following bleaching. Given the severity of

the 2015 bleaching event at Lanikai, which reached peak heat stress in September, it is possible that nine months may not have been sufficient for full reproductive recovery prior to our sampling in June 2016.

Presence of oocytes found in normal tissues in June 2016 were consistent with previous studies that found that reproductive activity was concentrated in normal tissue, with little or no reproduction by GAs (Yamashiro et al. 2000; Domart-Coulton et al. 2006; Work et al. 2008; Burns and Takabayashi 2011; Irikawa et al. 2011). However, in July 2017 I observed increased sizes and quantities of oocytes in the GA samples, far exceeding the oocyte presence in normal tissues in June 2016 or July 2017. This increased number and size of oocytes in GAs does not conform with previous findings in other scleractinian species (cited above), and suggests that GAs may not act the same across all taxa. Although I do not know why reproductive effort was greater in the GAs, one possible explanation is that GAs exhibited delayed oocyte development and spawning, such that the healthier portions of colonies spawned earlier (on full moon in June) and the GA portions of colonies spawned later in the summer (on full moon in July), as sampling took place 2 days before the full moon in July 2017. It is clear that more studies need to be done to elucidate the nature of GA reproduction.

In relating the lipid data to the reproductive data, I expected that levels of storage lipids would be higher during summer months when spawning normally occurs, because many species of coral oocytes are largely made up of WE (69-80%) and other storage lipids (Arai et al. 1993; Figueiredo et al. 2012; Lin et al. 2013). Even though gonad production was low in 2016, both total lipids and the functional lipid ratios (indicating more energy rich storage lipids) were high during summer months of 2016. In contrast to June 2016, gonad production was very high in July 2017 GAs and almost non-existent in normal samples, yet total lipids and functional lipid

ratios in GAs were low compared to normal samples. The only lipid class found in greater quantity in the GA tissue in July 2017 were structural PL. Lin et al. (2013) found high quantities of phosphatidyethanolamine and phosphatidylcholine, structural polar lipids, in the oocytes of five scleractinian coral species from Taiwan. If coral oocytes of *P. evermanni* contain mostly polar lipids, then functional lipid ratios might not differ between reproductive and non-reproductive tissue. The highest level of energy rich lipids occurred in normal tissue from 2016 that contained moderate amounts of oocytes. However, in the GAs from 2017, which contained the most oocytes comparatively, structural lipids dominated. One possibility is that the composition of oocytes produced in GAs is different than those produced in normal tissues and they therefore produce a different lipid signature. However, the lipid composition of coral oocytes varies a great deal among species and has yet to be determined for *P. evermanni.* Thus, it is unclear whether the differences between spawning seasons are attributed to differences between normal and GA tissue or other factors. However, if the oocytes produced in GAs are indeed anomalously low in energy rich lipids, this would likely have major implications for performance and survival of larvae produced from those eggs (Figueiredo et al. 2012).

Total lipids and functional lipid ratios as well as skeletal morphometric analysis all showed a consistent difference between GA and normal tissues in *Porites evermanni*, with larger calices, lower total lipid content, and less storage lipids in GAs. The inconsistency in seasonal variation in the reproductive data and some of the lipid responses illustrates a need for more studies on the differences between GAs and normal tissue over time and the seasonal variation of energy stores for massive *Porites* in Hawai'i. Our data suggest that understanding the recent history of both major environmental stressors and reproductive events will be important to interpreting the functional significance of lipid composition in scleractinian corals.

Acknowledgments

Funding for this study was provided by the National Science Foundation (OCE-1505158). I thank Shreya Yadav, Scott Goldberg, Erika Johnston, and Tara Hetz for assistance in the field, Thierry Work for assistance with histology analysis, and Lindsay Veazey for assistance with statistical analysis.

Tables and Figures

Table 4.1: P-values from multiple comparisons (with Tukey adjustments) for models of total lipids with health and date as factors, and health and date in combination with reproductive output.

Model without reproduction	August 2016	July 2017	August 2017
June 2016	p = 0.22	p = 0.001	p = 0.001
Model with reproduction	August 2016	July 2017	August 2017
June 2016	p = 0.004	p = 0.001	p = 0.001

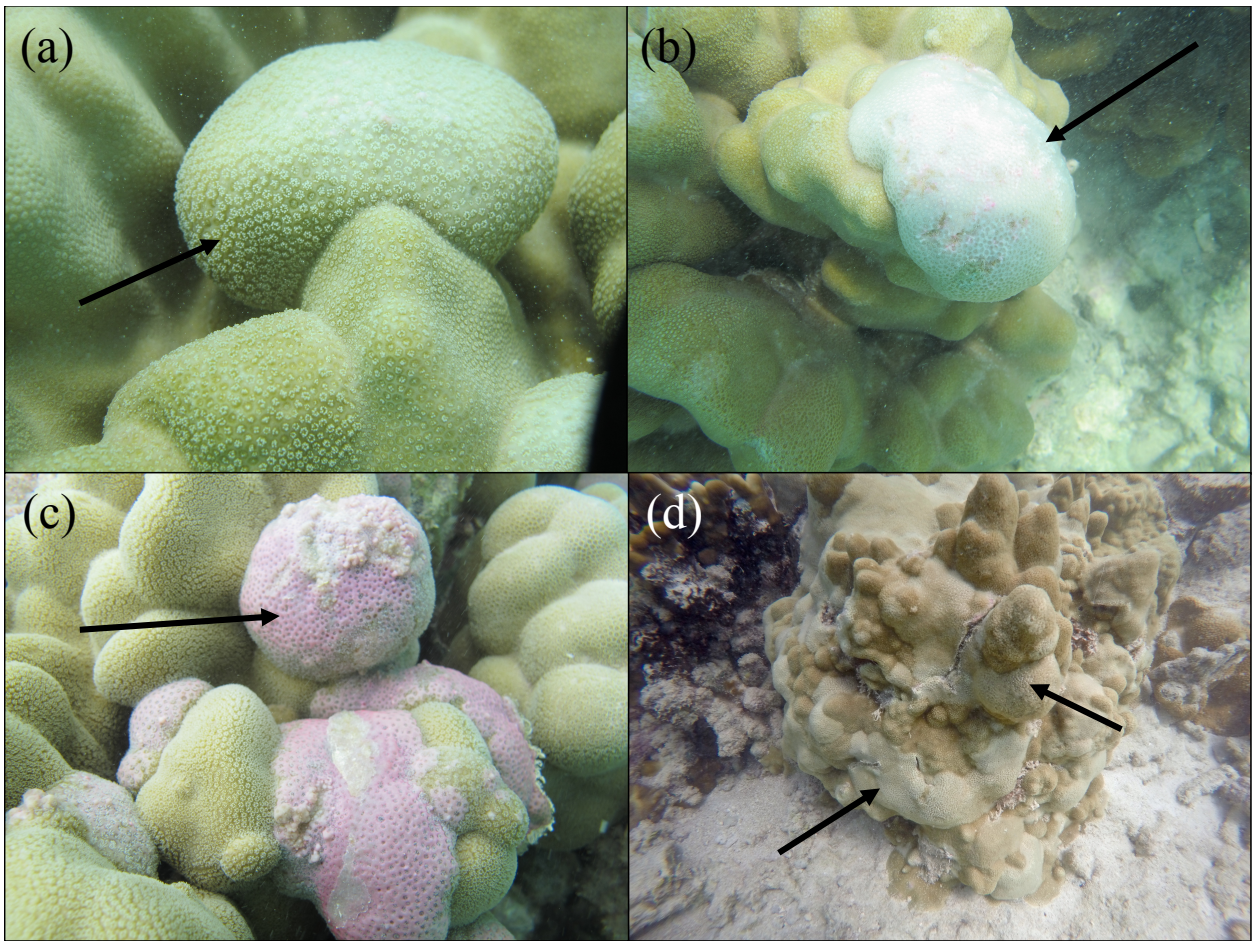

Figure 4.1: Examples of growth anomalies on two species of *Porites* at Lanikai, O‘ahu. (a) *P. evermanni* showing enlarged polyps, (b) *P. evermanni* showing pale coloration, (c) *P. evermanni* showing pink inflammatory response, and (d) *P. lobata* showing pale coloration.

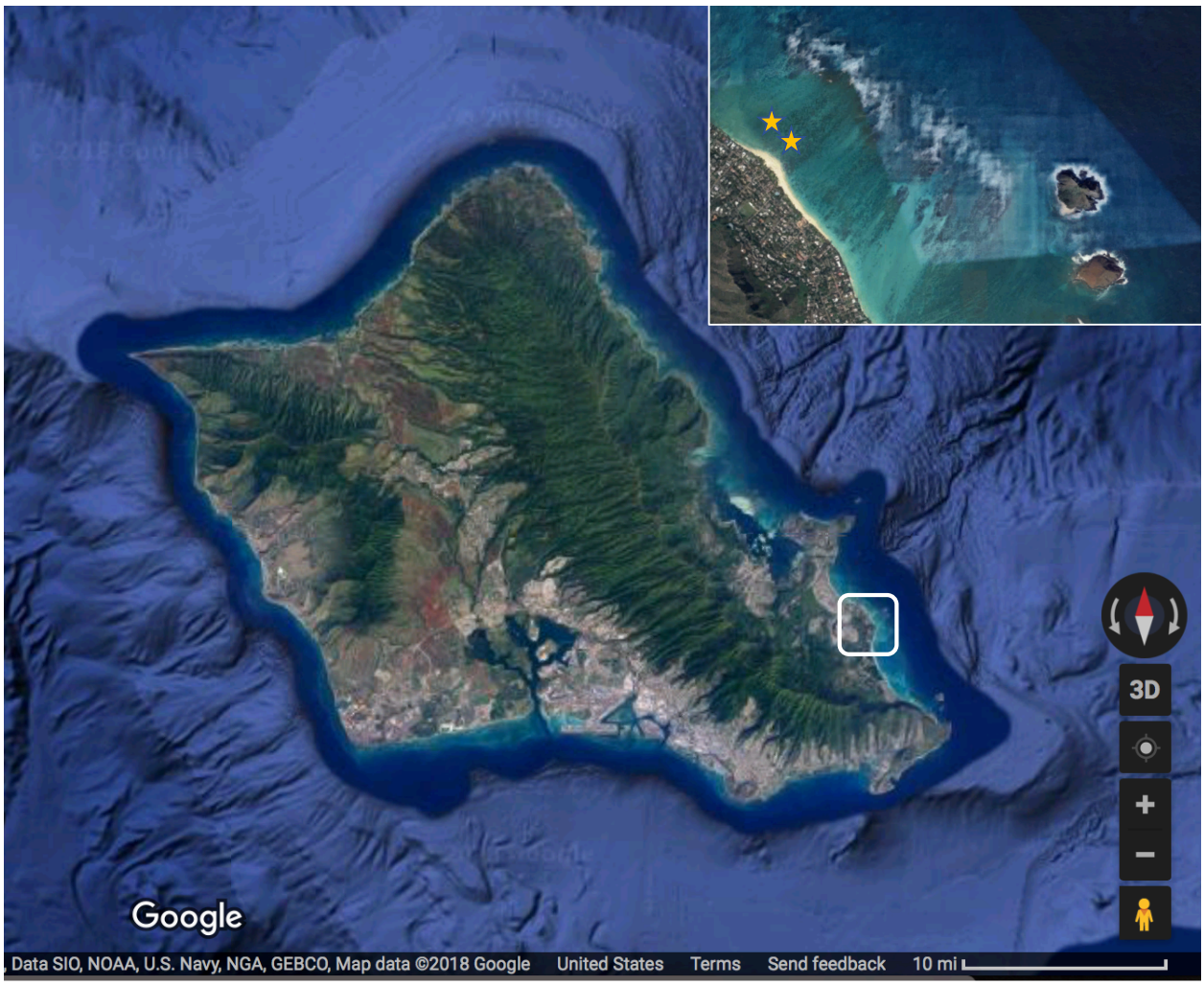

Figure 4.2: Reef sites where *P. evermanni* colonies were sampled at Lanikai, O‘ahu.

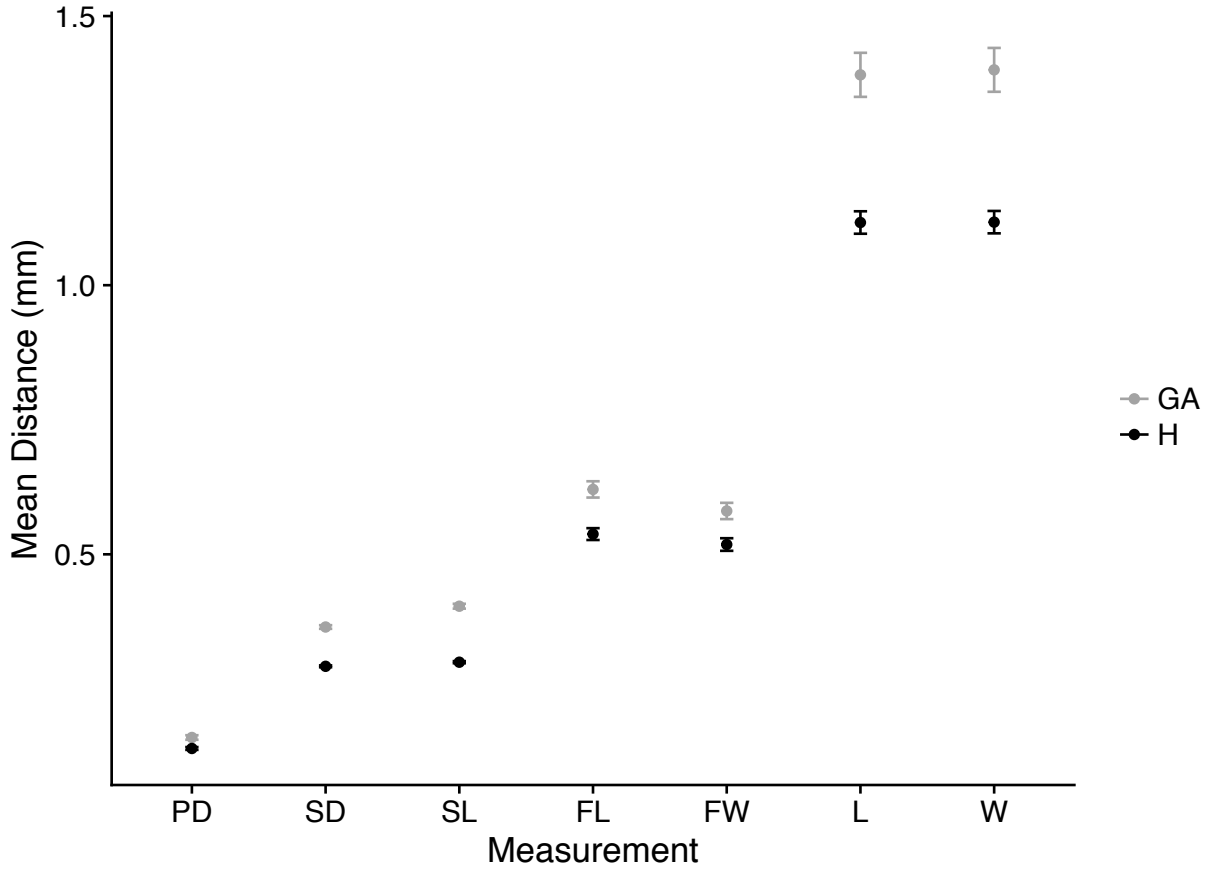

Figure 4.3: Mean distance (mm) (± se) for *P. evermanni* and *P. lobata* corallite morphometric traits (PD = pali distance, SD = septal distance, SL = septal length, FL = fossa length, FW = fossa width, L = length, W = width).

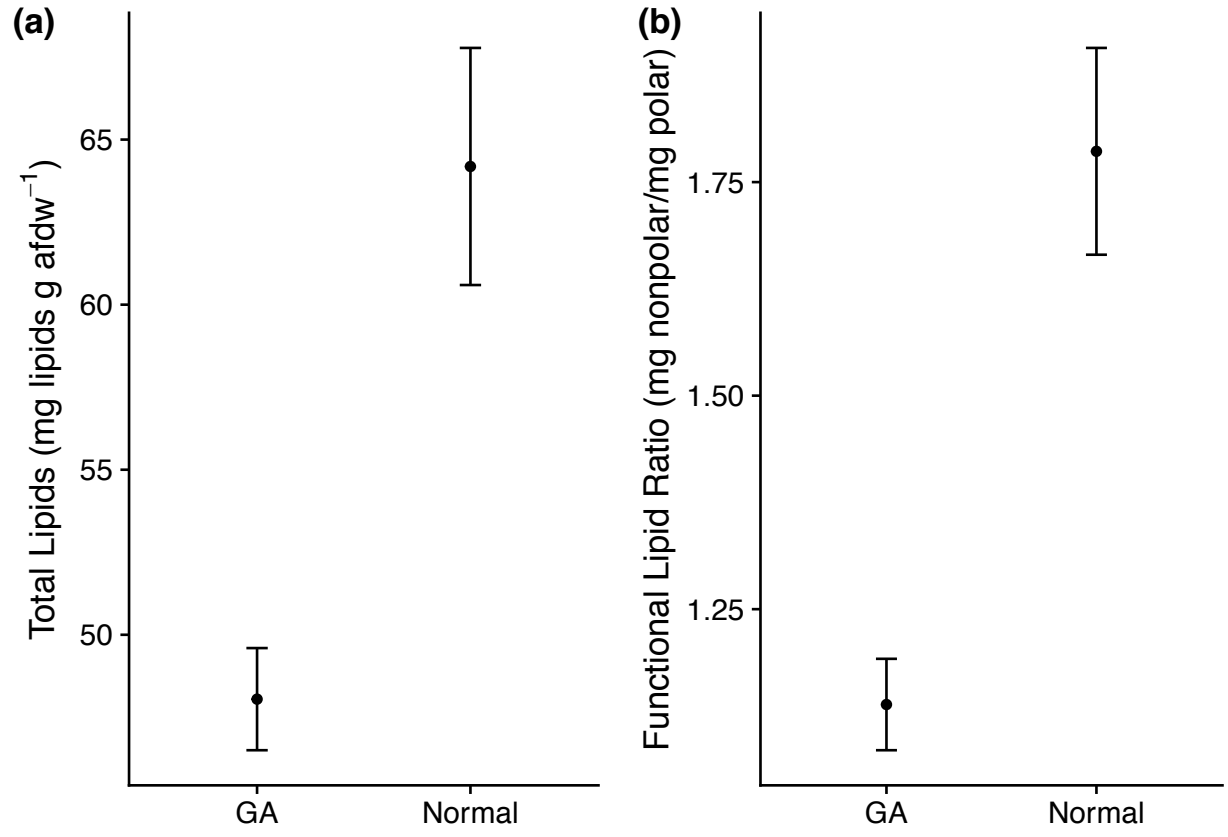

Figure 4.4: (a) Mean total lipids (mg lipid g afdw^{-1}) (± se) and (b) mean functional lipid ratios (mg non-polar/storage:mg polar/structural) (± se) for GAs and normal tissues from *P. evermanni* for all specimens combined across all dates (n = 76).

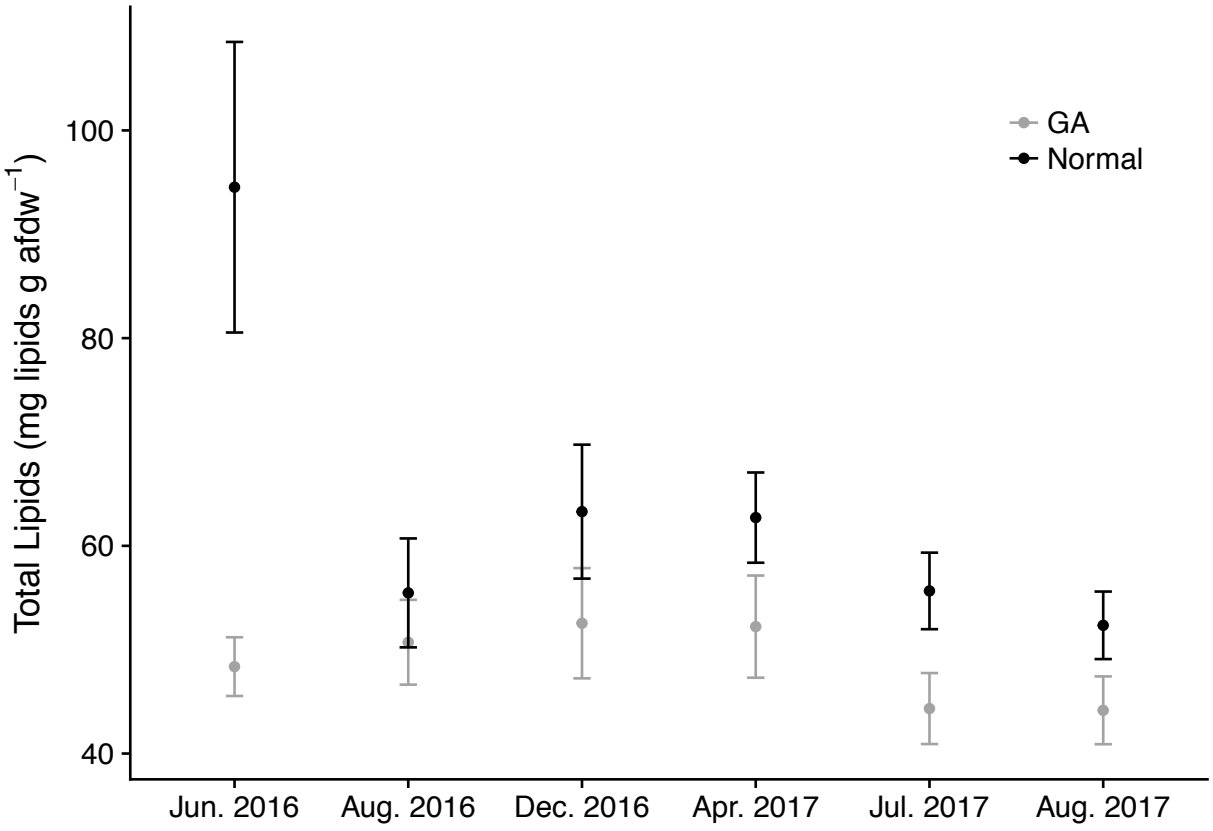

Figure 4.5: Mean total lipids (mg lipid g afdw^{-1}) (± se) for GAs and normal tissues of *P. evermanni* over two spawning periods in 2016/2017 (n = 15, except December and April n = 8).

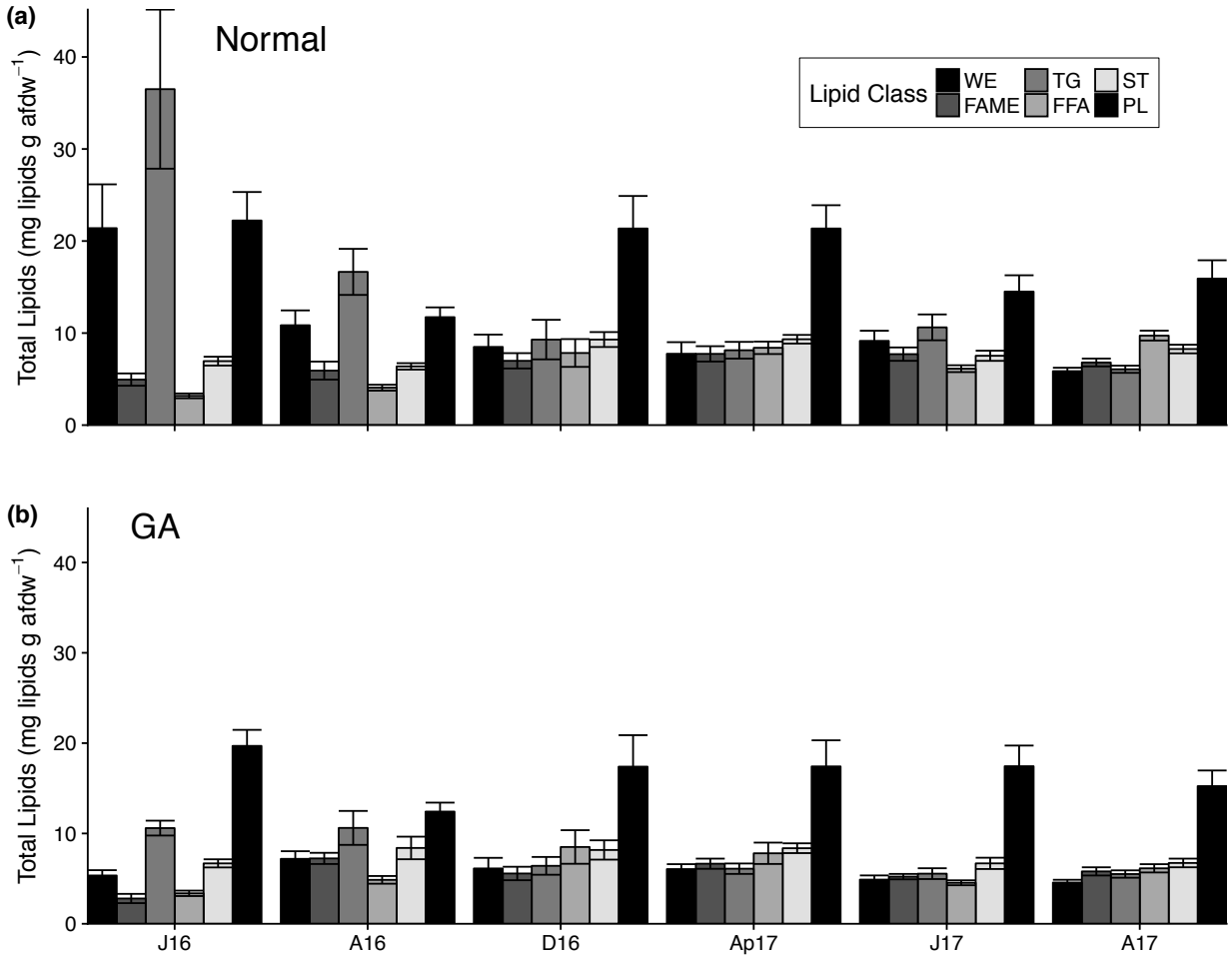

Figure 4.6: Mean total lipids (mg lipids g afdw^{-1}) (± se) for (a) normal and (b) GA tissue of *P. evermanni* for each lipid class (Nonpolar/storage: WE = wax esters, FAME = fatty acid methyl ester, TG = triacylglycerol, FFA = free fatty acids; Polar/structural: ST = sterol, PL = phospholipids) for June 2016, August 2016, December 2016, April 2017, July 2017, and August 2017 (n = 15, except December and April n = 8).

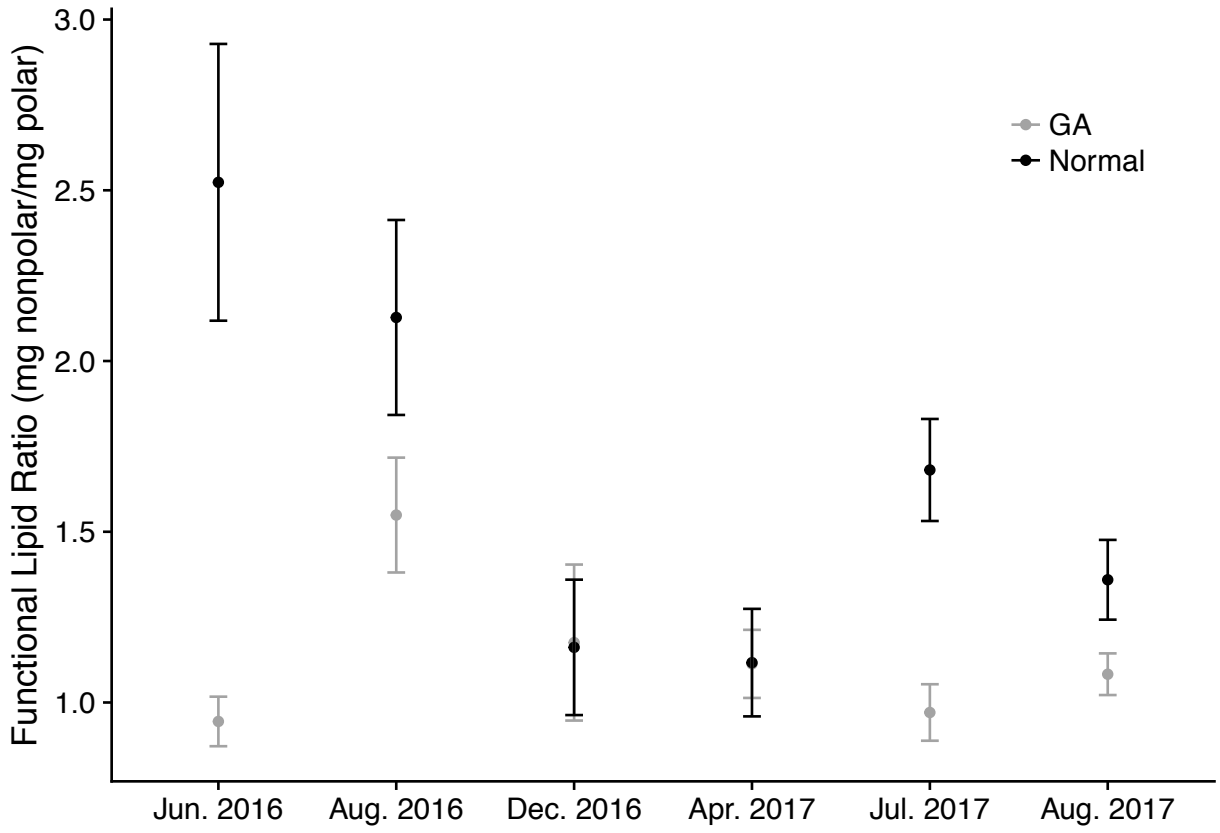

Figure 4.7: Mean lipid ratios (non-polar/storage:polar/structural) (± se) for GAs and normal tissues of *P. evermanni* over two spawning periods in 2016/2017 (n = 15, except for December and April n = 8).

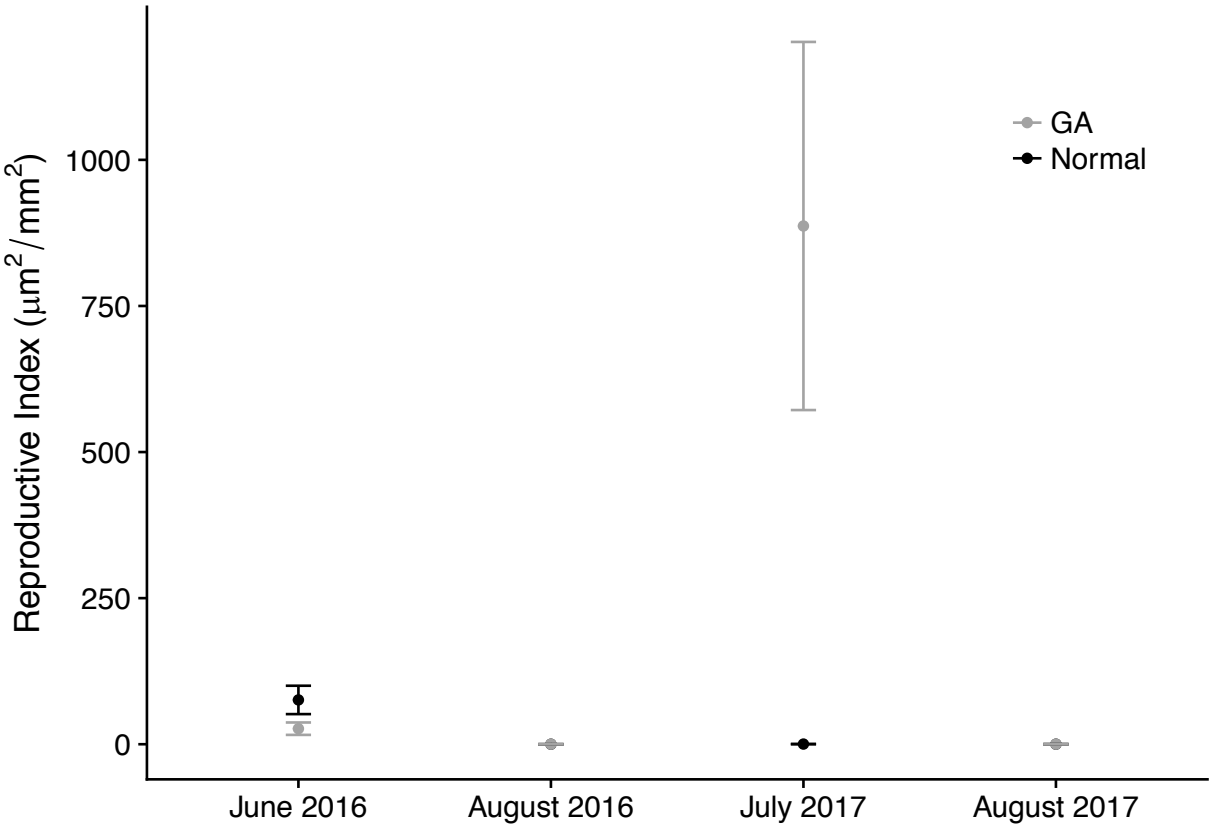

Figure 4.8: Mean reproductive index ($\mu m^2/mm^2$) (± se) for GAs and normal samples from *P. evermanni* colonies (n = 15) before (June 2016 and July 2017) and after (August 2016/2017) predicted spawning periods (full moon June-August).

CHAPTER FIVE

ADOPT A CORAL, SAVE A REEF: HOW CITIZEN SCIENCE AIDED IN THE ASSESSMENT OF BACK-TO-BACK BLEACHING EVENTS IN HAWAI‘I

Abstract

Citizen science is an area of rapidly expanding research, including its use in coral reef ecology and reef monitoring. Volunteers from a local community-run organization on O‘ahu, Hawai‘i assisted with a coral health monitoring project which captured and described back-to-back bleaching events that occurred in Hawai‘i in 2014 and 2015. Coral condition was assessed through two methods: scoring colony color using the CoralWatch Coral Health Chart (CHC) method and visual categorization of individual areas of each colony as healthy, bleached, and dead (professional assessment (PA) method). Volunteers assisted with the CHC method and were trained through a classroom-style lecture and in-water calibration exercise before collecting data for the project. Each volunteer was given a coral to “adopt” and follow over the course of the study. Bleaching was adequately described by the CHC method, closely matching the trends from the PA results for comparisons among species (*Porites evermanni*, *Porites lobata*, and *Pocillopora meandrina*) and between bleaching events. There were minor discrepancies between recovery descriptions from each method. Partial or complete coral mortality was not always directly related to the degree of pigment loss as assessed by the Coral Health Charts. The inclusion of relative condition in the PA method allowed for more specific inferences regarding coral colony health over time. In addition to providing a comparative assessment method, the involvement of citizen scientists in reef monitoring also provided a means for outreach and education within the community.

Introduction

The stress-induced loss of photosynthetic symbionts, known as bleaching, decreases coral energy supplies and can lead to partial or full mortality damaging overall reef health and ecosystem

function. Anthropogenically-induced climate change has resulted in an increase in conditions conducive to coral bleaching worldwide, most notably increased ocean temperatures. (Donner et al. 2005, 2009; Hughes et al. 2017). Corals provide the structural complexity that is needed to sustain the diversity and productivity of coral reef ecosystems, but with increasing threats the future of reefs may be at risk (Graham and Nash 2013).

Traditional scientific monitoring of reefs, particularly in reference to coral bleaching, often involves monitoring via transect surveys (i.e. visual, photo, video, aerial) (Glynn et al. 2001; McClanahan et al. 2001; Berkelmans et al. 2004; Maynard et al. 2008; Guest et al. 2012), or more specific and quantitative measurements of individual colony physiology and fitness (Brown et al. 2002a; Berkelmans and van Oppen 2006; Jones et al. 2008; Oliver and Palumbi 2011; Silverstein et al. 2015). While transect surveys provide useful data for understanding the impacts of such conditions *in situ*, and measurements of individual colonies provide more targeted details regarding fitness of individuals in a community, both method types often require considerable time, money, properly trained personnel, and if specimen collection is involved, agency-issued permits.

Citizen science (involving lay people in monitoring projects in conjunction with researchers who provide instruction and monitoring of data collection) can contribute to research efforts because well-designed projects alleviate time and resource limitations through gathering of data by volunteers using simple and revealing methodologies, while simultaneously expanding appreciation, understanding, and knowledge of scientific principles to community members. Whether for monitoring programs or in hypothesis-driven research, the use of citizen science in research has been increasing in the field of conservation biology in the past two decades including its application in the field of coral reef ecology (Darwell and Dulvy 1996; Delaney et

al. 2008; Goffredo et al. 2010; Azzurro et al. 2013; Vianna et al. 2014; Branchini et al. 2015; Galvis and Galvis 2016; Raoult et al. 2016). While there is some skepticism within the scientific community regarding the efficacy of data collected from volunteers (i.e., Dickinson et al. 2010; Kosmala et al. 2016) an increasing body of peer-reviewed publications utilizing volunteer-collected data sets demonstrates how properly crafted volunteer tasks can be beneficial to the advancement of scientific research

With a number of successful and widespread reef monitoring projects already established in Hawai‘i (e.g. Reef Check and Eyes on the Reef), I sought to test a concept for developing a monitoring program involving individual coral colonies through time using a coral health card as a tool. The Coral Health Chart developed for the CoralWatch program (Siebeck et al. 2006) is based on changes in symbiont density and chlorophyll *a* concentration in thermally-stressed corals. The gradient of hues on the card is meant to accurately reflect the changes in pigmentation during the bleaching process, with the goal of providing a low-cost and easy-to-learn tool that can adequately report bleaching presence and severity for common reef building corals (Siebeck et al. 2006; Reid et al. 2009; Marshall et al. 2012; Raoult et al. 2016). Monitoring individual colonies as opposed to general reef health allows more insight into coral community dynamics as well as provides volunteers the chance to witness the seasonal changes on a reef from a fixed perspective. The aim of this research was to create a method for volunteers to collect data on an individual colony scale through time via an “adopt a coral” program and evaluate the results of these findings in understanding bleaching dynamics in comparison to a more quantitative monitoring methodology.

Methods

This study was conducted at A‘alapapa Reef (offshore from Lanikai Beach), located on the windward side of the island of O‘ahu. Six sites were established within the lagoon along the 2.4 kilometer stretch of the reef in June of 2014 (Figure 5.1).

Individual colonies of *Porites evermanni* (25), *Porites lobata* (5), and *Pocillopora meandrina* (10) were selected at each site. Corals were monitored weekly from August-December 2014 and bi-monthly from January-December 2015. Coral health was assessed in two ways, first through the Coral Health Chart (CHC) method in collaboration with local residents of Lanikai. Working with a neighborhood-based organization, Malama Ka‘ōhao, volunteers were recruited to participate in an “adopt a coral” program. This program aimed at assisting in monitoring coral health throughout the predicted bleaching events in Hawai‘i from August 2014-November 2014. To participate, volunteers were required to attend a training that included an educational lecture regarding coral and coral reefs, global climate change, coral bleaching, and briefing them on the method for the monitoring program. The remainder of the training involved an in-water calibration exercise to practice monitoring coral colonies utilizing the Coral Health Chart (CHC) as well as to calibrate assessments between volunteers (Figure 5.2a-c). The CHC displays four color hues to accommodate species differences; each hue contains colored squares scaled from one to six with one representing a bleached coral and six representing a fully pigmented coral (Figure 5.3). Not all coral species in Hawai‘i fit perfectly into one hue category or another, so the emphasis for volunteers was placed on scale (1-6) rather than hue (B, C, D, E). Volunteers were instructed to record the color score on the CHC that matched the pigmentation of the particular coral colony for both the lightest area on the colony and the darkest area on the colony. Each volunteer was given specific corals at a select site to monitor once a week and were

provided with datasheets for consistent data entry. Of the 40 designated colonies for this study, 26 were monitored by the 6 volunteers and the remaining 14 colonies were monitored by myslef using the same CHC method. The other method involved assessment of coral health through estimations of relative tissue condition in three categories: healthy, bleached, and dead. This method will hereafter be referred to as the professional assessment (PA) method. All 40 colonies were monitored via the PA method in order to compare the bleaching trends of the same colonies between the two datasets. In addition to these two survey methods, photographs were taken of each colony with a marked PVC stake and color card included for size and color reference.

These colony-level data from both survey methods were split into two approximately 9-month periods encompassing before, during, and after each bleaching event. These periods are referred to here as the 2014 event (August 2014 - March 2015) and the 2015 event (April 2015 - December 2015). To address pseudoreplication and temporal autocorrelation, the data for individual colonies were compressed into two calculated metrics for each colony to examine bleaching and recovery for each event. A bleaching quotient was calculated for the PA data as the maximum percentage of bleached tissue over the initial percentage of healthy tissue (B_{max}/H_i), and for the CHC data as the lowest light area color score over the initial dark area color score ($Light_{min}/Dark_i$). Because the bleaching values for the two methods were operating on opposite scales (100 % bleached vs. CHC score of one), the bleaching metric for the CHC data was subtracted from one to match the scale of the PA data ($1 - Light_{min}/Dark_i$). A recovery quotient was calculated for the PA data as the final percentage of healthy tissue over the initial percentage of healthy tissue (H_f/H_i), and for the CHC data as the final dark area color score over the initial dark area color score ($Dark_f/Dark_i$). Kruskal-Wallis rank sum tests were performed on

each set of bleaching and recovery metrics (CHC data and PA data) to compare patterns of bleaching and recovery between species and between thermal stress events.

Results

Bleaching

The corals at A‘alapapa Reef bleached heavily in Fall of both 2014 and 2015 and both the PA and CHC results recorded this through the examination of coral health over time (Figure 5.4). When examining bleaching trends by species, the CHC method (Figure 5.4c) performed well in comparison to the PA method (Figure 5.4a) with both showing *Pocillopora and Porites* bleaching heavily and *P. evermanni* bleaching more than *P. lobata*. When compared statistically, a Kruskal-Wallis test revealed significant differences in bleaching susceptibility between species for both methods (CHC: p-value < 0.001, PA: p-value = 0.025). When examining bleaching trends by thermal stress event, the CHC method (Figure 5.4d) and the PA method (Figure 5.4b) showed a decrease in bleaching overall in 2015 as compared to 2014; however, the PA method showed a larger difference between years than the CHC method. A Kruskal-Wallis test examining bleaching trends by thermal stress event showed significantly less bleaching in 2015 than 2014 for the PA results (PA: p-value<0.001), while the CHC results showed no significant difference in bleaching between events (CHC: p-value = 0.014). When examining the bleaching trends for species by event, the CHC method revealed slight differences in the degree of bleaching between years for *P. evermanni* and *P. meandrina*, with the CHC data showing smaller differences between years for both species (Figure 5.5). For *P. lobata* the CHC results displayed a similar pattern to the PA data of less bleaching in 2015.

Recovery

While bleaching at A‘alapapa Reef was severe during both 2014 and 2015 thermal stress events and some partial and full mortality did occur, colonies largely recovered after both events. Overall, the CHC method underperformed in describing recovery trends in comparison to the PA method (Figure 5.6). When examining recovery trends by species, the CHC method did well in tracking the recovery of *P. evermanni* and *P. meandrina*, but failed to pick up the lack of recovery in *P. lobata* (Figure 5.6a, c). When compared statistically, a Kruskal-Wallis test found a significant difference in recovery patterns between species for both the PA data and the CHC data (PA: p-value < 0.001, CHC: p-value = 0.015). When examining recovery trends by thermal stress event, the CHC method and the PA method both showed similar recovery efforts both years with slightly less recovery in 2015 (Figure 5.6). There was a slightly larger difference between years in the PA data and the recovery between years was significant different (p = 0.016; Figure 5.6d), but there was no significant difference in recovery between years in the CHC data (p-value = 0.165; Figure 5.6b). The CHC method performed poorly compared to the PA method when comparing the recovery trends for species split by event (Figure 5.7). The trend of lower *P. evermanni* recovery in 2015 versus 2014 was portrayed by both datasets, however the trends for both *P. lobata* and *P. meandrina* showed differences. *P. lobata* showed limited recovery with less recovery in 2015 by the PA data (Figure 5.7a), but the CHC data documented substantial recovery for both years (Figure 5.7b). Both methods indicated recovery was high overall for *P. meandrina*. However, the CHC data for *P. meandrina* showed a trend of higher recovery in 2015 (Figure 5.7b), and the PA results indicated that *P. meandrina* experienced less recovery in 2015 (Figure 5.7a).

Discussion

This study demonstrated how citizen science monitoring was a reasonably successfully tool for monitoring coral health on an individual colony scale, and successfully involved and informed local communities about important threats to reef health. The CHC method was sufficient in comparison to more quantitative assessments in evaluating bleaching and recovery patterns for three major reef building species in Hawai‘i, but further improvements to the CHC method could improve the accuracy of assessments.

Overall the 1-100% scale of the PA method allowed for more specific inferences regarding bleaching and recovery in comparison to the six-unit CHC scale. However, comparisons of these two methods were generally congruent, with bleaching patterns similar for all three species. The two methods showed slight discrepancies in bleaching trends when individual species were parsed by event, and there were minor differences in the amount of bleaching in 2014 between the two events themselves. While the relative differences between events varied between methods, the overall trend of less bleaching in the 2015 event was the same.

Recovery patterns described for both coral health survey methods showed similarities in species trends as a whole for *P. evermanni* and *P. meandrina*, but differed for *P. lobata*. The colonies of *P. lobata* monitored throughout the duration of this study experienced severe partial mortality following the bleaching event in 2014, leading to reductions in their recovery relative to the other species monitored. The CHC data did not show this trend, but instead indicated successful recovery for these colonies. This discrepancy lies in the lack of incorporation of relative tissue health into the CHC method, which focused on coral pigment levels as the sole determinant of health. This difference in *P. lobata* recovery between methods likely accounted

for the inconsistency in the overall recovery trend between events. When comparing the recovery trends for each species parsed by event the CHC method showed discrepancies in comparison to the PA method for *P. lobata*, with over-representation of recovery in 2014. Differences in recovery between methods for *P. meandrina* were present but indicated high recovery for those colonies both years regardless of the differences between years.

Overall, the CHC method, which was partially utilized by citizen scientists to collect data for a period of the study, performed adequately in terms of describing bleaching and recovery trends for the monitored colonies at A‘alapapa Reef. The differences observed in bleaching and recovery trends in the CHC data could be avoided with improvements to the methodology. I ascribe the majority of the discrepancy issues with the CHC method evaluating coral health through two scores, with no weight given to relative health values. The PA method did take into account relative tissue health, but also involved the estimation of health values for each category. Estimation is often an attribute withheld from citizen science methods due to inconsistencies associated with collecting this type of data from non-technically trained individuals. Given this, for improvement on this methodology in the future, I would suggest utilizing one score for each colony instead of two (lightest area and darkest area). I believe that if volunteers scored each colony based on the majority of the tissue on that colony it would help eliminate the issues from providing two scores without estimation of their relative weight. Additionally, having volunteers take photos on each survey date would allow for double checking their assessment of coral health and provide an image database of monitored colonies through time. Furthermore, development of a Coral Health Chart for Hawaiian corals would make future monitoring via health charts more accurate with colors and pigment hues created based on common corals found in Hawai‘i.

Volunteer retention and turnout was highly variable, however, and as such the results represent the potential problem with citizen science projects in the absence of professional scientists using the same method (in this case the CHC). However, I believe this study presents a framework for future citizen science reef monitoring and offered successful educational outreach to the local communities surrounding the A‘alapapa Reef system. The volunteers who did participate in the project fueled the interest of conservation-minded individuals from the community group Malama Ka‘ōhao and the greater Lanikai community. Their efforts, in addition to our work, led to two science outreach events hosted by the community. At each event, I spoke about the project, presented results, and shared insights about the health of the coral on the reef (Figure 5.2d). Other scientists and managers working in the community spoke or set up tables and posters to talk about their efforts to conserve reef resources and promote sustainable and safe ocean activities. With the health of coral reefs at risk today, any event or project that can inspire others to make more sustainable choices or volunteer for conservation causes is a worthwhile endeavor. Citizen science can make a favorable impact on the scientific community and on the communities where science takes place. With the future of the oceans in jeopardy, it has never been more important to inspire others to make changes for the betterment of the environment and citizen science accomplishes this while still collecting scientific data. Moving forward we need to identify the scientific questions that need answering which citizens can assist with in a way that better impacts their communities and their reefs.

Acknowledgements

I thank Katherine Courtney and Lee Bell for assistance with setting up the community monitoring program and for assistance in the field. I also thank Alan White, Vangie White, Derek Esibil, Marya Grambs, and Susan Bryan for their participation as citizen scientists.

Figures

Figure 5.1: Monitoring sites along the shallow Aʻalapapa reef flat off Lanikai Beach, Oʻahu, Hawaiʻi.

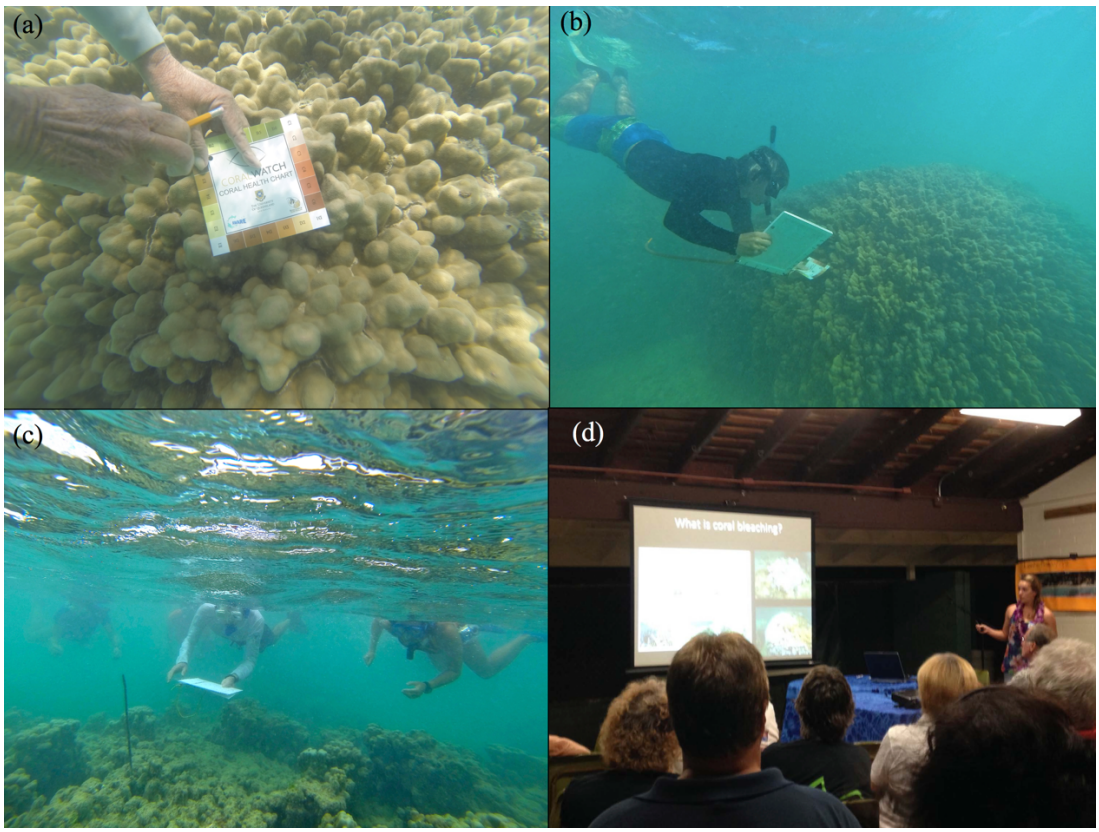

Figure 5.2: (a-c) Community training to use the CoralWatch Coral Health Chart (d) community outreach presentation on coral bleaching and the results of the study following the first bleaching event (2014).

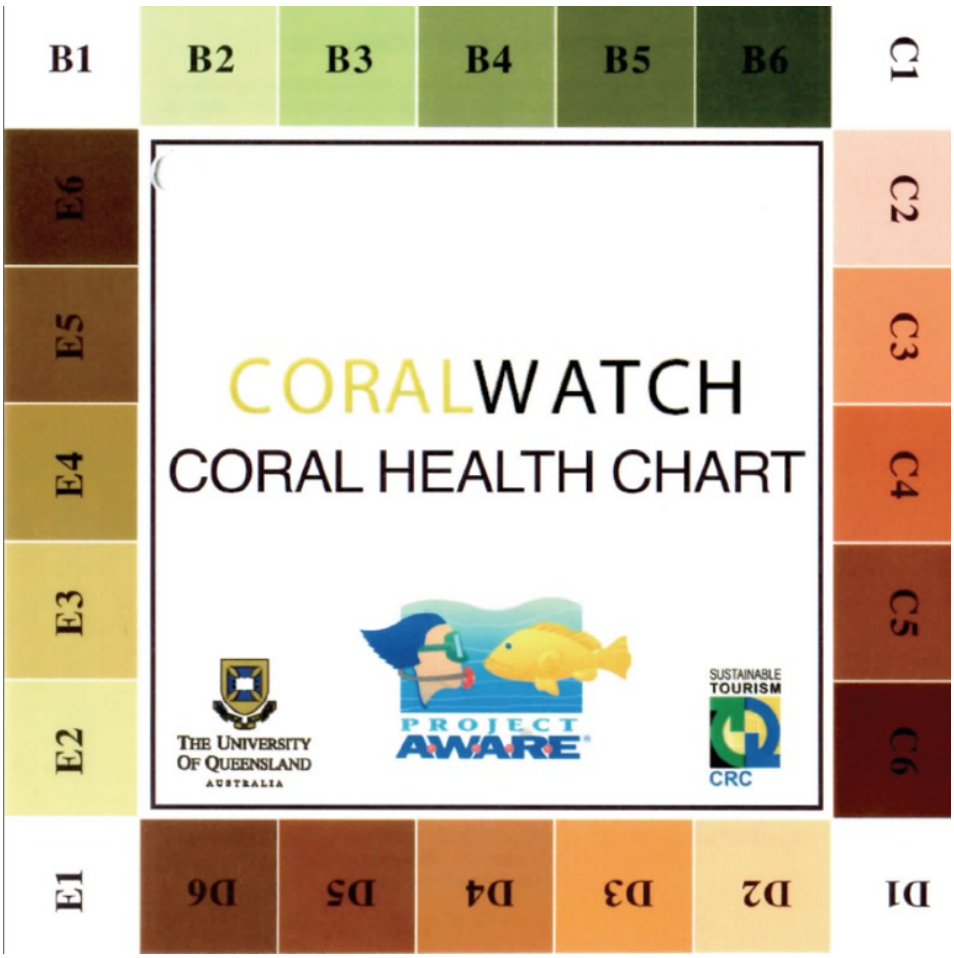

Figure 5.3: CoralWatch Coral Health Chart with four hues (B-E) and a scale from 1-6 representing differences in coral chlorophyll *a* and *Symbiodinium* concentrations during the bleaching process (Siebeck et al. 2006).

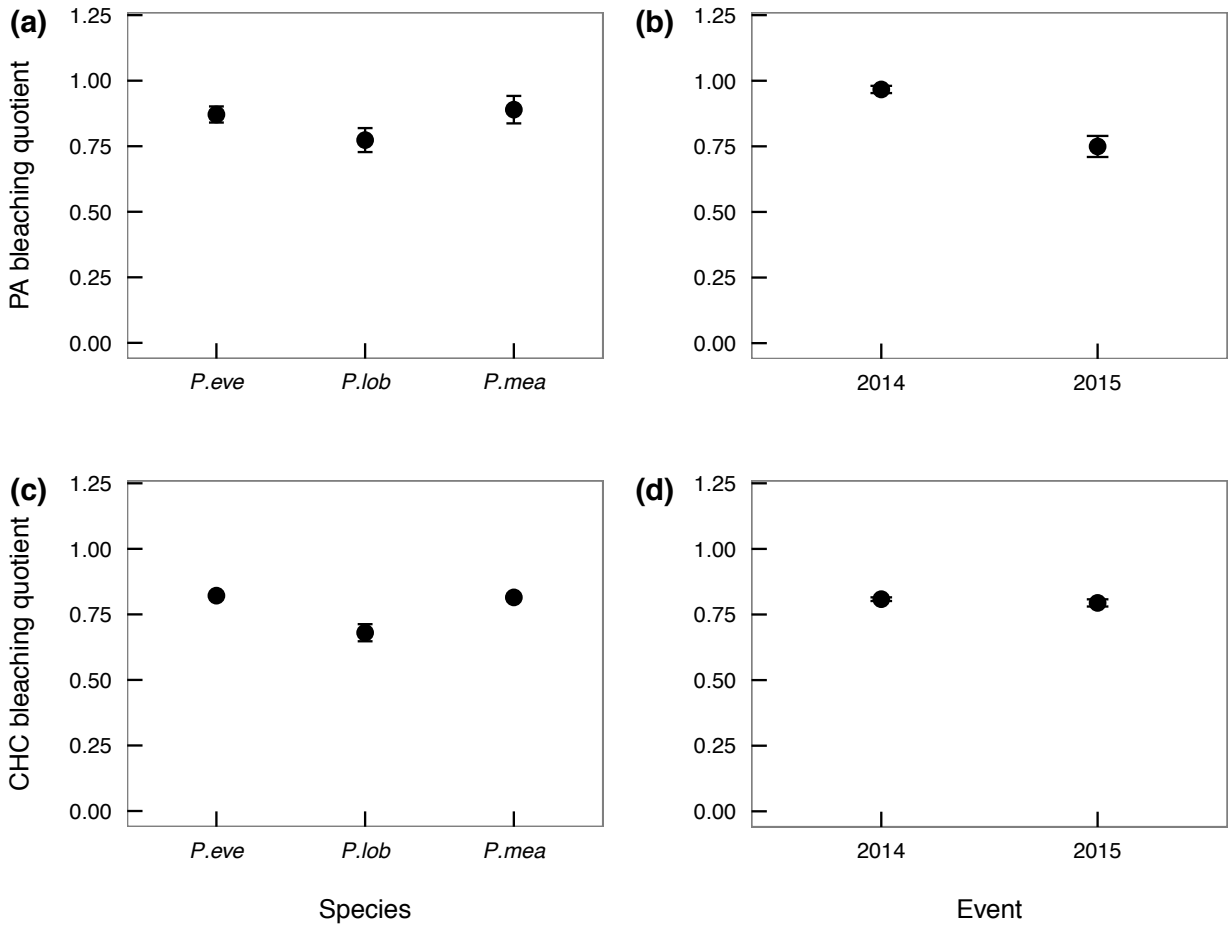

Figure 5.4: Bleaching quotients (± SE) for the (a & b) professional assessment method (PA) data (B_{max}/H_i) and the (c & d) Coral Health Chart (CHC) data ($Light_{max}/Dark_i$) by species (*Porites evermanni*, *P. lobata*, and *Pocillopora meandrina*) and for each bleaching event.

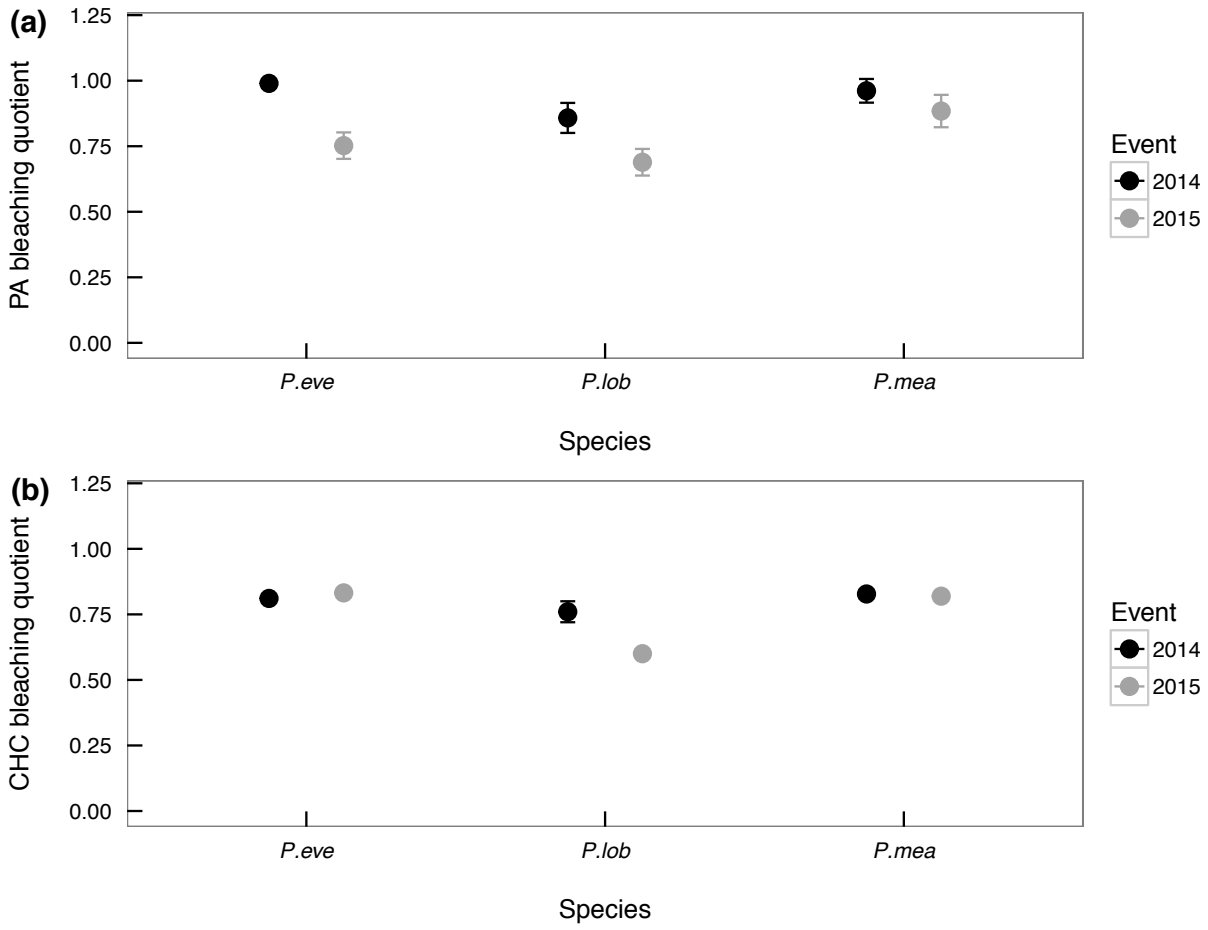

Figure 5.5: Bleaching quotients (± SE) for the (a) professional assessment method (PA) data (B_{max}/H_i) and the (b) Coral Health Chart (CHC) data ($Light_{max}/Dark_i$) for *Porites evermanni*, *P. lobata*, and *Pocillopora meandrina* by bleaching event.

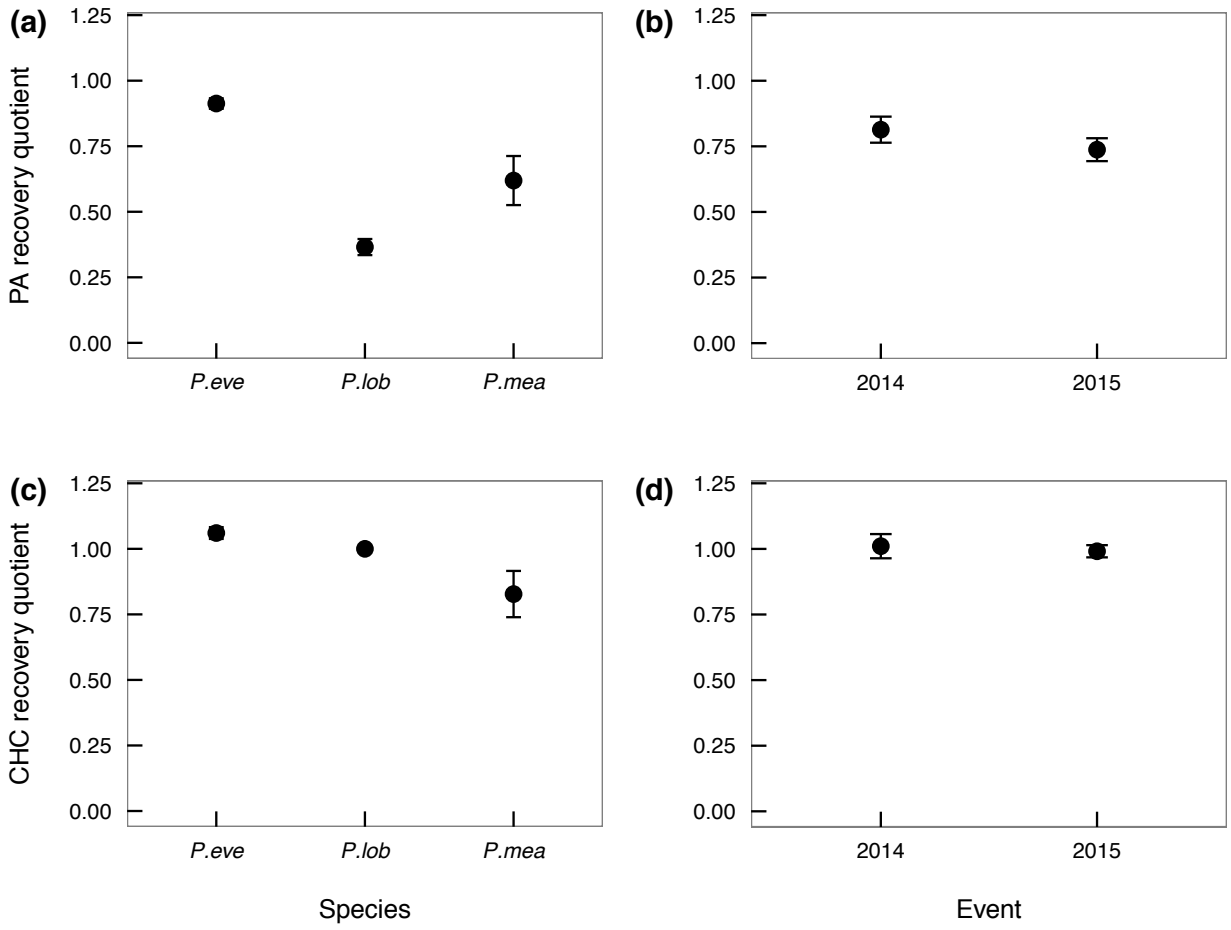

Figure 5.6: Recovery quotients (± SE) for the (a & b) professional assessment method (PA) data (H_f/H_i) and the (c & d) Coral Health Chart (CHC) data ($Dark_f/Dark_i$) by species (*Porites evermanni*, *P. lobata*, and *Pocillopora meandrina*) and for each bleaching event.

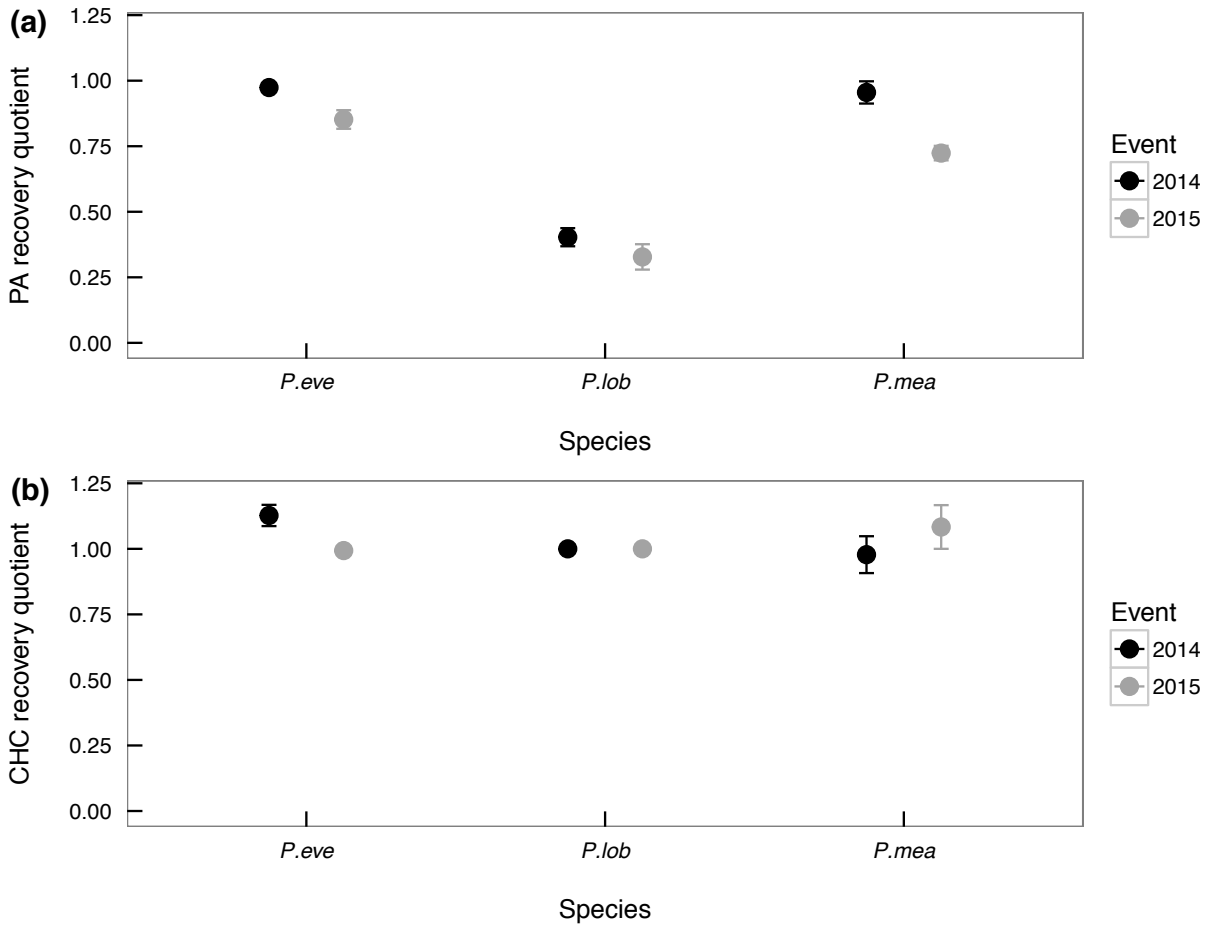

Figure 5.7: Recovery quotients (± SE) for the (a) professional assessment method (PA) data (H_f/H_i) and the (b) Coral Health Chart (CHC) data ($Dark_f/Dark_i$) for *Porites evermanni*, *P. lobata*, and *Pocillopora meandrina* by bleaching event.

CHAPTER SIX

CONCLUSIONS

Overview of Dissertation Objectives

I will conclude this dissertation by giving a summary of the dissertation objectives I set out to accomplish at the beginning of this work and a brief description of how I met those objectives, what the main conclusion were, and finally suggestions for future research. The overall goal of this dissertation was to better describe and characterize coral bleaching on Hawaiian reefs through a number of more pointed research questions:

1) *What were the inter-specific and intra-specific bleaching patterns at Lanikai in 2014 and 2015, and was there any evidence of acclimatization between years?*

The results of this chapter successfully described the patterns of bleaching, recovery, and mortality at Lanikai in both 2014 and 2015 by measuring both colony level and reef scale processes. The bleaching events that occurred in the Main Hawaiian Islands in 2014 and 2015 provided an unprecedented natural experiment to compare bleaching dynamics across the same reef scape two years in a row, experiencing different levels of *in situ* thermal stress each year.

Altogether bleaching patterns were similar to previous observations in the literature: massive *Porites* and branching *Pocillopora* colonies experienced extensive bleaching, with less bleaching in 2015 compared to 2014, and the encrusting *Montipora* colonies experienced moderate bleaching in 2015. Colonies of *P. evermanni* recovered well both years, but the colonies of *P. lobata* surprisingly did not recovery well, especially compared to the response of *P. evermanni*. Colonies of *Pocillopora* either fully recovered or did not recover at all. All colonies of *Montipora* recovered well. There was little partial mortality seen both years in P. evermanni, and both *Montipora* species. All colonies of *P. damicornis* and a few of *P. meandrina* died, and *P. lobata* colonies experienced large amounts of partial mortality in 2014. In addition, the strength of utilizing colony level bleaching, recovery, and mortality descriptions

allowed for more specific and informed nuances about overall reef health across time and between bleaching events. The *in situ* degree heating weeks calculated showed Lanikai experienced significantly more thermal stress in 2015 compared to 2014 and more thermal stress both years compared to the NOAA Coral Reef Watch metric. When bleaching data and thermal stress data were compared I showed that the corals successfully acclimatized to conditions of thermal stress within one year, as the same individual colonies bleached less the second year despite greater thermal stress.

In the future, research during bleaching events should incorporate both *in situ* measurements and post-hoc physiological analysis to determine a more quantitative measure of health in physiological terms. I believe this chapter presents compelling evidence to support the incorporation of colony scale bleaching work in future coral reef research as it provides more power to comparative analysis when partial mortality and post disturbance selection are taken into consideration. Additionally, I believe this work shows the importance of understanding the local environment through *in situ* measurements of environmental variables.

2) *Were there genomic differences between differentially bleached Montipora capitata next to each other on the reef?*

The results of this chapter successfully described the genomic differences in differentially bleached individuals of *M. capitata* located near each other on the same reef. The results found no strong genomic differences between the two groupings, but found population structure between all Windward Oʻahu populations sampled. Lanikai and Kāneʻohe Bay were the most different from each other, and Waimānalo was most similar to Kāneʻohe Bay. The signal of population structure may have clouded the discrimination of phenotypic groupings or the method

utilized did not accuratly capture the variation in the genome. If there was indeed no genetic differences between bleached and non-bleached individuals, this might be because Hawai‘i is an isolated location with little history of thermal stress prior to 2014.

Observations of starkly contrasting bleaching phenotypes on reefs are a rising interest in the coral bleaching community. Molecular tools, in particular the use of –omic methods are rising in popularity due to the decreasing cost of analysis and the incredible amounts of data these methods provide. These methods allow for more accessible ways to answer complex questions and this chapter of dissertation research is one of the first to investigate differential bleaching phenotypes using these molecular techniques. While a lack of power and small sample size limited the inferences I could make from this dataset, this chapter provides a starting point from which future studies can build on in attempt to better understand what the underlying molecular mechanisms are that determine an individual's response to thermal stress. Future work could better execute a paired design and retain much larger sample sizes to strengthen the power of the downstream analysis.

3) *Are growth anomalies (GAs) of Porites evermanni morphologically and physiologically different?*

The results of this chapter successfully described some of the morphological and physiological differences between GAs and the surrounding normal tissue through examination of corallite structure, lipid composition, and reproductive effort. Corallites of both *P. evermanni* and *P. lobata* GAs had larger corallites measurements. GAs of *P. evermanni* on average had less lipids overall, particularly less energy rich storage lipids, but also more structural lipids when compared to normal tissue. GAs are known to have faster growth rates and were likely utilizing

the storage lipids to grow and creating structural lipids to provide support to new cells and membranes. Previous bleaching stress likely affected overall lipid values as well as reproductive output as there was little gonad material found in 2016. Surprisingly, the most gonads (in particular eggs) were found in the GA samples in 2017. The difference in timing of tissue sampling could have been one reason for the disparity in trends between years, or there is a biological difference in the spawning cycles of the normal and GA parts of a colony. The lipids data did not correlate with the reproductive data which suggested either that the eggs of GAs are not made up of a lot of lipids, or that the eggs produced in GAs aren't viable. This chapter showed for the first time that GAs of some species are reproductive and represent a possibility that either the GAs are stealing resources from the host colony to reproduce, or that the GAs are not maladaptive as previously thought. This is the first study examining the lipid composition and reproductive output of *P. evermanni* GAs and as such adds a new perspective to the coral disease and GA literature.

In the future, research looking more specifically at the reproductive nature of the GAs as well as the timing of reproduction should sample at more frequent and consistent time points across multiple years. In addition, comparison of all metric with colonies that are visibly healthy without GAs is necessary to establish a better understanding of how detrimental GAs are or are not to their host colony.

4) How well did a citizen science reef monitoring project describe reef health and bleaching?

The results of this chapter successfully compared the data from a citizen science reef monitoring program to the data from Chapter 2. While not perfect, the citizen science data did a good job of describing bleaching and recovery of coral colonies at Lanikai when compared to the other

monitoring method. This work allowed for working with the community and helped connect my dissertation work to the people who utilize the reefs on a daily basis. This chapter in particular allowed me to incorporate outreach and education abut coral reefs and their threats from climate change into the work.

In the future, bleaching monitoring methods for citizen scientists could utilize more geographically appropriate coral cards, as this is something currently being developed for Hawaiian corals. Overall, this doctoral dissertation research adds a number of important datasets to the existing research of corals in Hawai‘i, as well as the coral bleaching process, and presents evidence of hope for the future as the impacts of climate change on our oceans continue to increase.

Manuscript Publication and Author Acknowledgements

Chapter 2: *What doesn't kill you makes you stronger: evidence for acclimatization in corals during repeated natural thermal stress.* Chapter 2 will be submitted to Marine Ecology Progress Series.

Authors: Massey TM, Marko PM, Oliver, TA, Hunter CL

Chapter 3: *Genomic analysis of differential bleaching responses to identical thermal stress in Montipora capitata in Hawai'i.* Chapter 3 will be submitted to Molecular Ecology.

Authors: Massey TM, Hunter CL, Oliver TA, Marko PM

Chapter 4: *Lipid storage and reproduction in coral growth anomalies in Porites evermanni.*

Chapter 4 will be submitted to Marine Ecology Progress Series.

Authors: Massey TM, Hunter CL, Hong C, Moran AL

Chapter 5: *Adopt a coral, save a reef: how citizen science aided in the assessment of back-to-back bleaching events in Hawai'i.* Chapter 5 has been submitted to PeerJ and is in review.

Authors: Massey TM, Hunter CL

APPENDIX A: SUPPLEMENTAL TABLES

Table S2.1: P-values of multiple comparisons of bleaching quotient patterns for the fixed effect of species (*Porites evermanni*, *Porites lobata*, *Pocillopora meandrina*, *Montipora capitata*, and *Montipora patula*).

	P.eve	*P.lob*	*P.mea*	*M.cap*
P.lob	0.386			
P.mea	1.00	0.501		
M.cap	0.737	0.966	0.829	
M.pat	**<0.001**	0.472	**0.002**	0.068

Table S2.2: P-values of multiple comparisons of recovery quotient patterns for the fixed effect of species (*Porites evermanni*, *Porites lobata*, *Pocillopora meandrina*, *Montipora capitata*, and *Montipora patula*)

	P.eve	*P.lob*	*P.mea*	*M.cap*
P.lob	**0.003**			
P.mea	**<0.001**	0.999		
M.cap	0.801	**0.001**	**<0.001**	
M.pat	0.889	**0.001**	**<0.001**	0.995

Table S2.3: P-values of multiple comparisons of mortality susceptibility patterns for the fixed effect of species (*Porites evermanni*, *Porites lobata*, *Pocillopora meandrina*, *Montipora capitata*, and *Montipora patula*).

	P.eve	*P.lob*	*P.mea*	*M.cap*
P.lob	**0.022**			
P.mea	**0.003**	1.00		
M.cap	0.862	**0.015**	**0.005**	
M.pat	0.753	**0.006**	**0.001**	1.00

Table S2.4: P-values for multiple comparisons for the fixed effect of species (*Porites compressa*, *Porites evermanni*, *Porites lobata*, *Pocillopora meandrina*, *Montipora capitata*, and *Montipora patula*) on the amount of tissue affected (bleached + pale + mucus sheets) for all video transects regardless of date.

	P.comp	*P.eve*	*P.lob*	*P.mea*	*M.cap*
P.eve	**<0.0001**				
P.lob	**<0.0001**	**<0.0001**			
P.mea	**<0.0001**	**<0.0001**	**<0.0001**		
M.cap	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	
M.pat	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Table S2.5: P-values for multiple comparisons for the fixed effect of video transect dates on the amount of tissue affected (bleached + pale + mucus sheets) regardless of species, with ecologically significant comparisons highlighted in boxes.

	14-Sep	14-Oct	15-Jan	15-Mar	15-May	15-Jul	15-Sep	15-Oct
14-Oct	**<0.0001**							
15-Jan	<0.0001	<0.0001						
15-Mar	<0.0001	<0.0001	<0.0001					
15-May	<0.0001	<0.0001	0.065	0.0001				
15-Jul	<0.0001	0.023	<0.0001	<0.0001	<0.0001			
15-Sep	0.343	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		
15-Oct	<0.0001	**<0.0001**	<0.0001	<0.0001	<0.0001	<0.0001	**<0.0001**	
15-Dec	<0.0001	<0.0001	<0.0001	0.996	0.005	<0.0001	<0.0001	<0.0001

Table S2.6: P-values for multiple comparisons for the fixed effect interaction of species (*Porites compressa*, *Porites evermanni*, *Porites lobata*, *Pocillopora meandrina*, *Montipora capitata*, *Montipora patula*) with year for the amount of tissue affected (bleached + pale + mucus sheets) for video transects in September and October of 2014 and 2015.

	P.com (2014)	*P.eve* (2014)	*P.lob* (2014)	*P.mea* (2014)	*M.cap* (2014)	*M.pat* (2014)
P.com (2015)	0.382					
P.eve (2015)		**<0.0001**				
P.lob (2015)			0.143			
P.mea (2015)				**0.003**		
M.cap (2015)					1.00	
M.pat (2015)						**<0.0001**

Table S4.1: X-Y corallite coordinates used to measure the 40 morphometric traits analyzed.

Name	Points	Description
SL1	1:2	Septa length
SL2	3:4	Septa length
SL3	5:6	Septa length
SL4	7:8	Septa length
SL5	9:10	Septa length
SL6	11:12	Septa length
SL7	13:14	Septa length
SL8	15:16	Septa length
SL9	17:18	Septa length
SL10	19:20	Septa length
SL11	21:22	Septa length
SL12	23:24	Septa length
SD1	1:3	Septa distance
SD2	3:5	Septa distance
SD3	5:7	Septa distance
SD4	7:9	Septa distance
SD5	9:11	Septa distance
SD6	11:13	Septa distance
SD7	13:15	Septa distance
SD8	15:17	Septa distance
SD0	17:19	Septa distance
SD10	19:21	Septa distance
SD11	21:23	Septa distance
SD12	23:1	Septa distance
PD1	2:4	Pali distance
PD2	4:6	Pali distance
PD3	6:8	Pali distance
PD4	8:10	Pali distance
PD5	10:12	Pali distance
PD6	12:14	Pali distance
PD7	14:16	Pali distance
PD8	16:18	Pali distance
PD9	18:20	Pali distance
PD10	20:22	Pali distance
PD11	22:24	Pali distance
PD12	24:2	Pali distance
FW	20:8	Fossa width
FL	2:14	Fossa length
W	7:19	Width
L	1:13	Length

APPENDIX B: SUPPLEMENTAL FIGURES

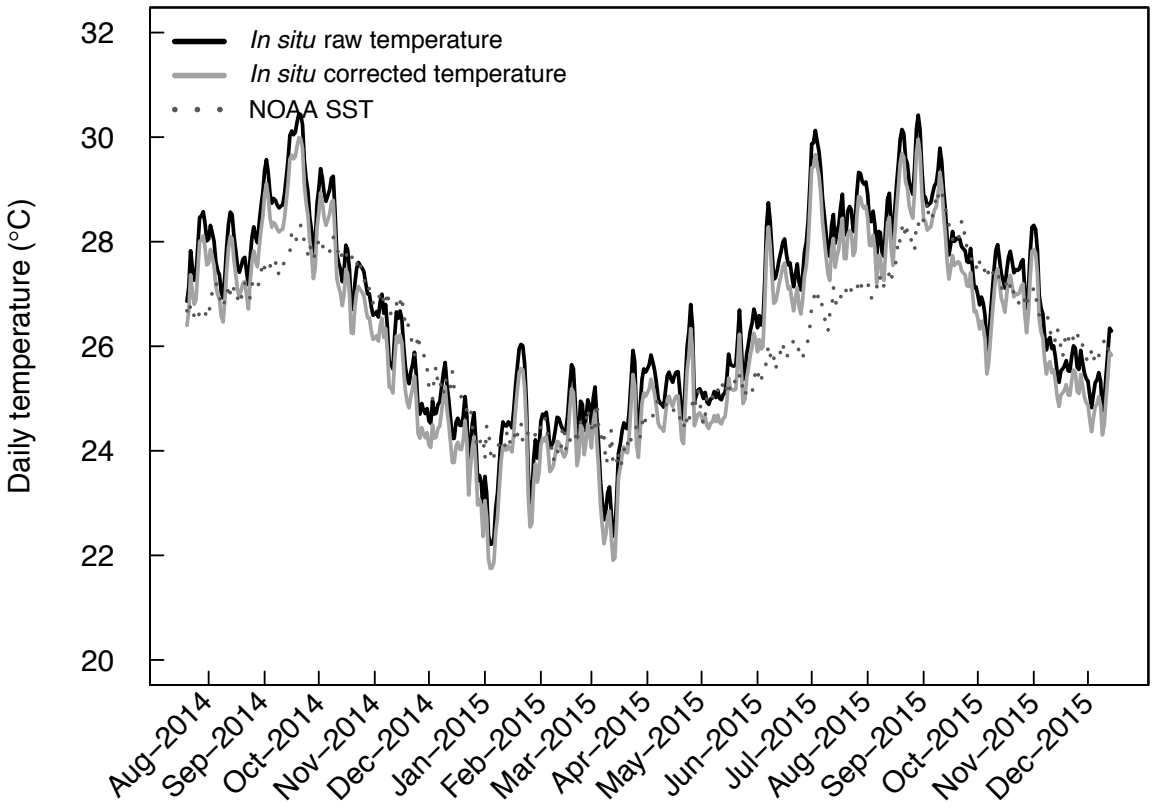

Figure S2.1: Comparison of *in situ* and corrected *in situ* sea water temperatures and NOAA Coral Reef Watch 5km satellite nighttime sea surface temperature product (NOAA Coral Reef Watch 2013a) for A‘alapapa Reef from July 20, 2014 – December 15, 2015.

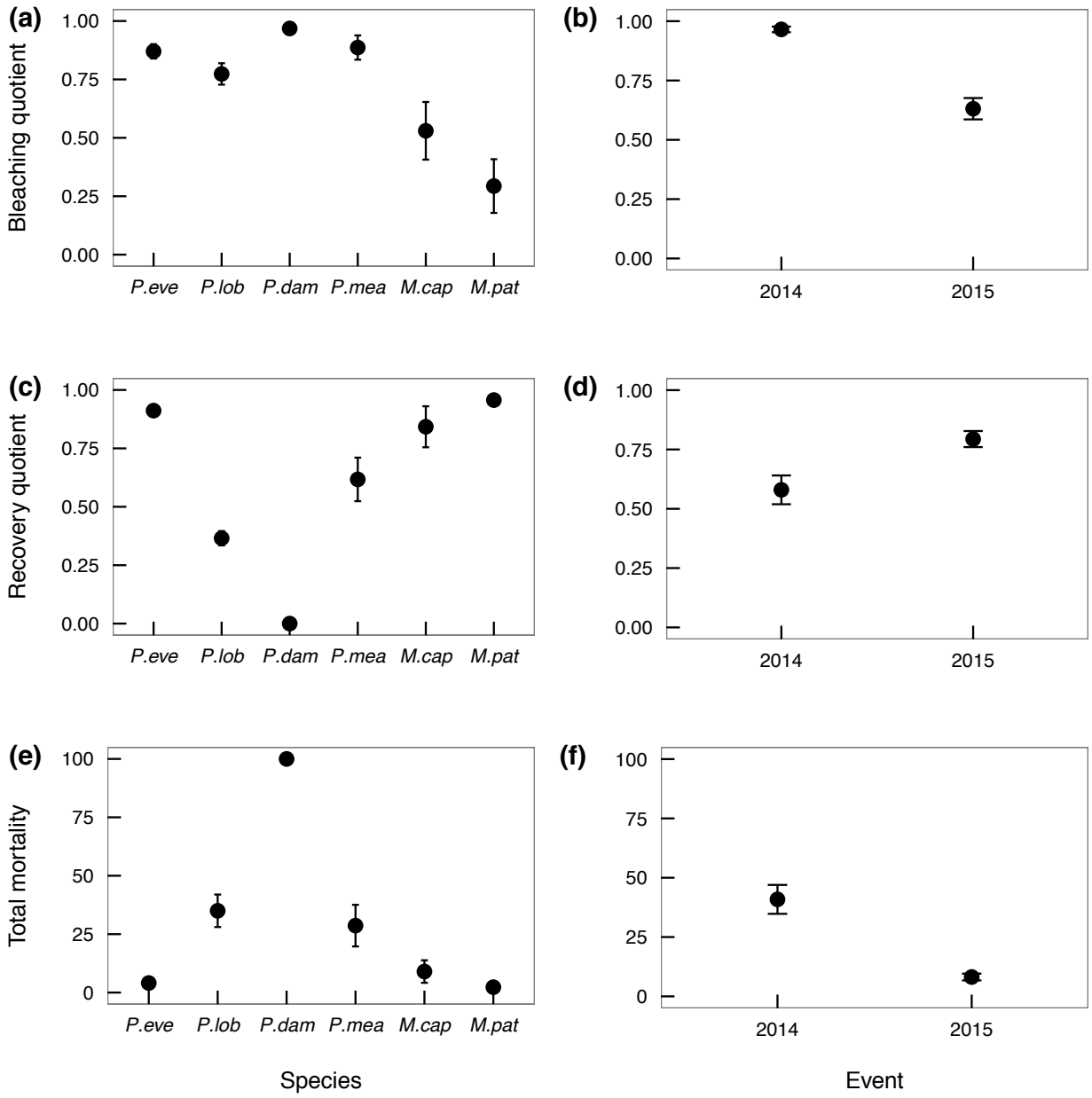

Figure S2.2: (a) Mean bleaching quotients ($B_{max}/H_i \pm SE$), (c) mean recovery quotients ($H_i/H_f \pm SE$), (e) and mean mortality values ($M_f - M_i \pm SE$) for all *Porites evermanni*, *Porites lobata*, *Pocillopora damicornis*, *Pocillopora meandrina*, *Montipora capitata*, and *Montipora patula* for both events, and (b, d, f) with all species combined for each bleaching event.

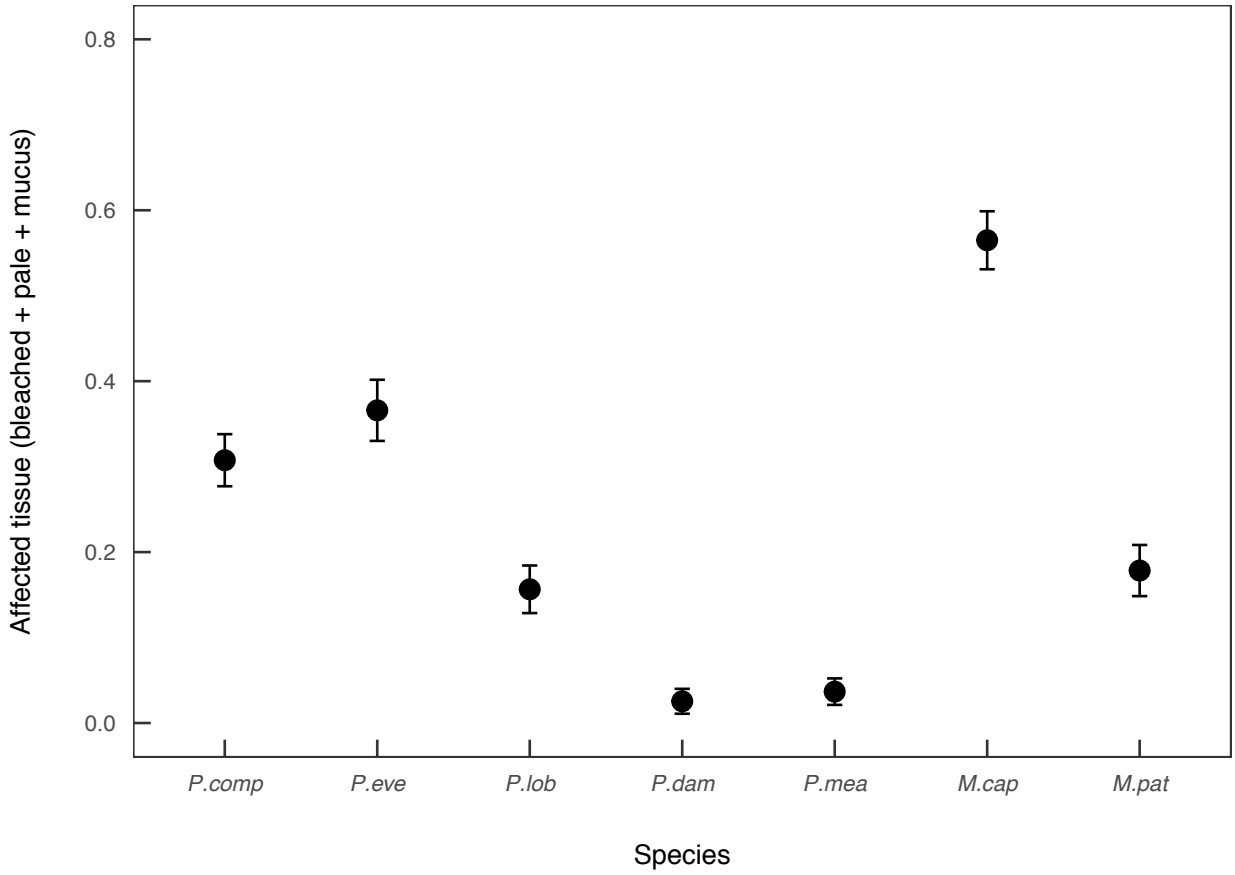

Figure S2.3: Mean affected tissue (bleached + pale + mucus sheets) (± SE) on all 13 video transect dates (2014-2015) for *Porites compressa*, *Porites evermanni*, *Porites lobata*, *Pocillopora damicornis*, *Pocillopora meandrina*, *Montipora capitata*, and *Montipora patula*.

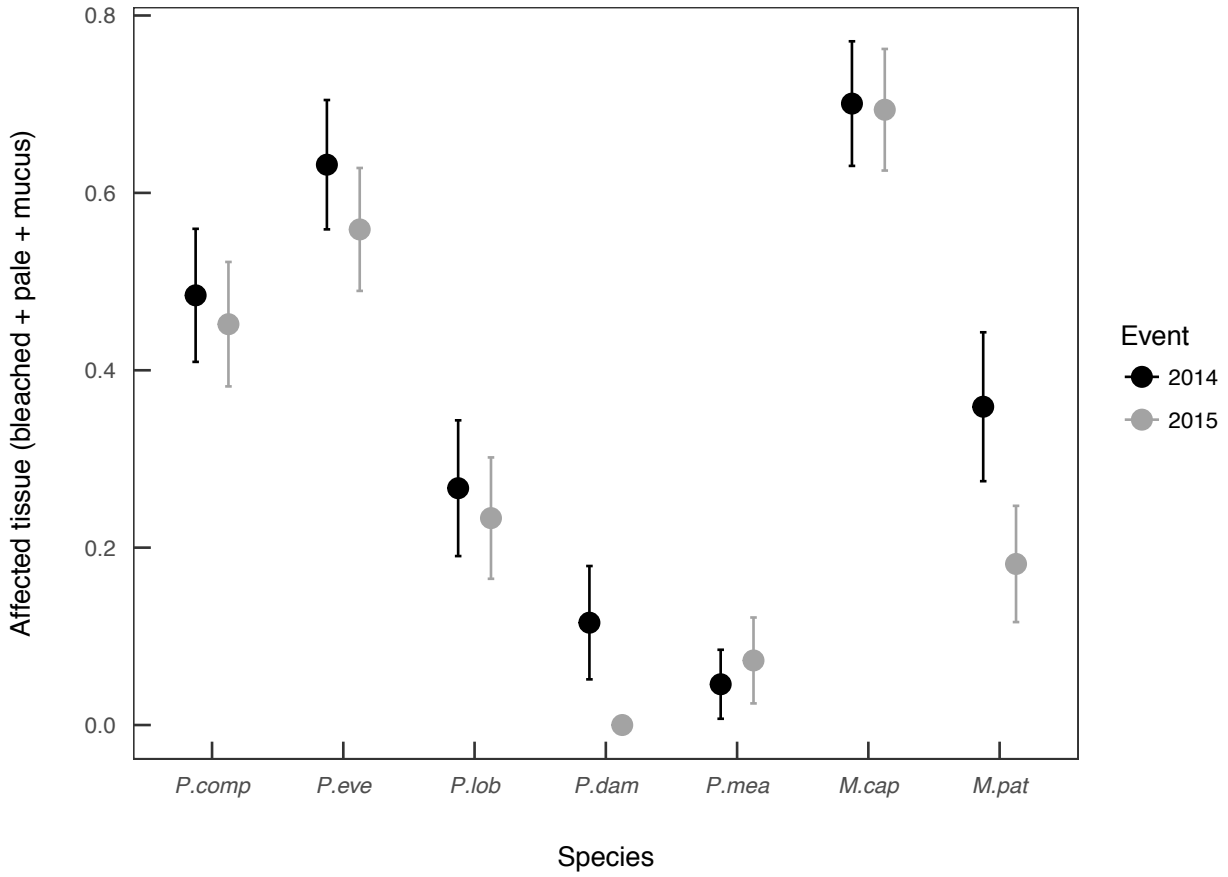

Figure S2.4: Mean affected tissue (bleached + pale + mucus sheets) (± SE) for *Porites compressa*, *Porites evermanni*, *Porites lobata*, *Pocillopora damicornis*, *Pocillopora meandrina*, *Montipora capitata*, and *Montipora patula* for video transects in September and October of 2014 and 2015.

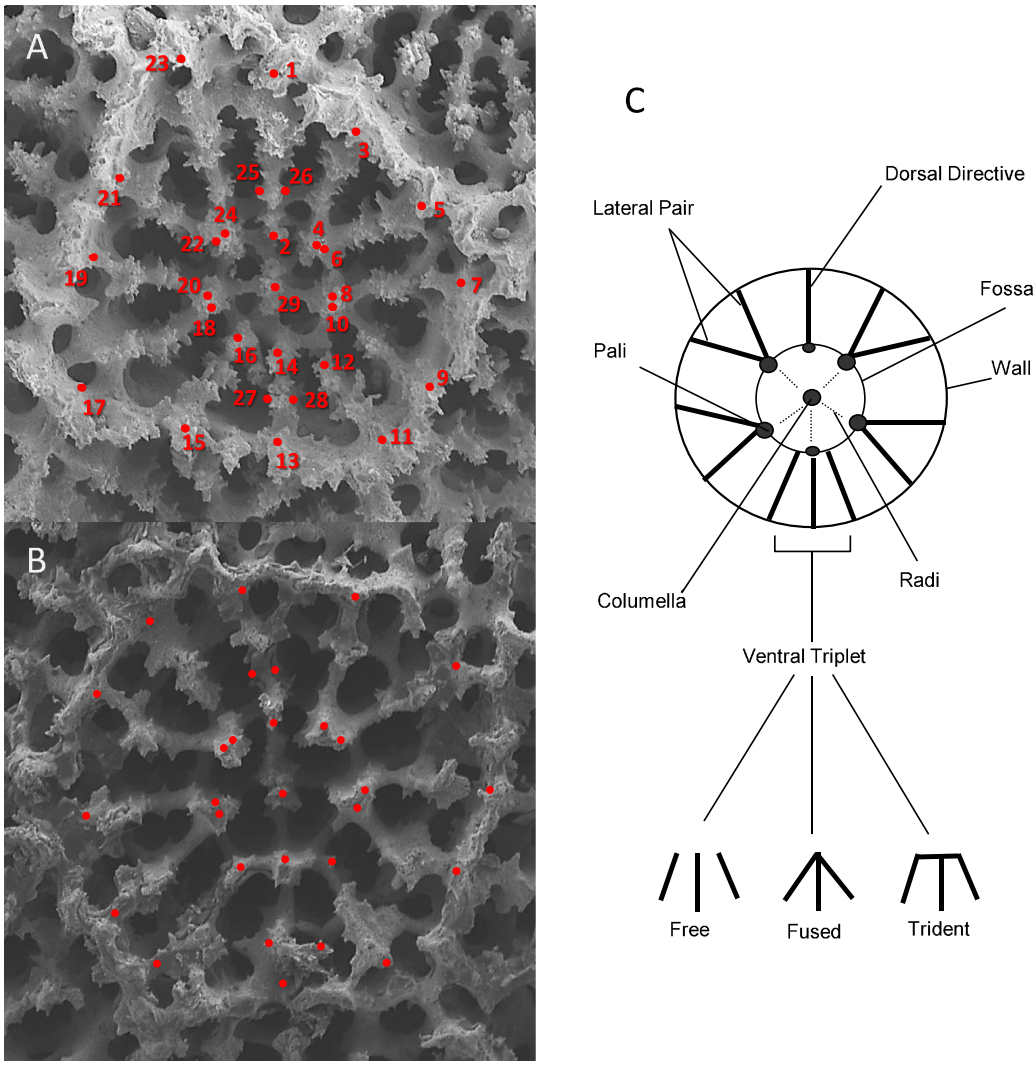

Figure S4.1: Corallite X-Y coordinate points numbered on (a) an SEM image of a *P. lobata* normal corallite, (b) a *P. evermanni* normal corallite, and (c) a diagram showing major diagnostic components (copyright Forsman et al. 2015).

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